European Commission

VOLUME 3- Annex B (A14111B)

- CHLOROTHALONIL -

B.9 Ecotoxicology data

Rapporteur Member State: The Netherlands

October 2016

Renewal Assessment Report and Proposed decision of the Netherlands prepared in the context of the possible approval of chlorothalonil under Regulation (EC) 1107/2009

Version history page

Date	Version history
May 2016	Draft Renewal Assessment Report
August 2016	Initial Renewal Assessment Report
August 2017	Revised Renewal Assessment Report according to the comments in the Evaluation Table. Revisions are in yellow, except for typo's which are not marked.
October 2017	Revised Renewal Assessment Report according to the expert consultation Revisions are in green, except for typo's which are not marked.

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B.9 Ecotoxicology data and assessment of risks for non-target species

Study summaries and evaluations and their respective conclusions were adjusted by RMS when deemed necessary, except where indicated otherwise. Where the study evaluation or conclusion from RMS deviated from the applicant's, this is reported and explained in the text, as well as in the boxed conclusion. Studies from the old dossier were not re-evaluated, and summary/evaluations from the old DAR/Addendum were copied, except where indicated otherwise. If on top of the study summary/evaluation no reference is made to the old dossier, then it concerns a new study submitted for this Annex I renewal. In the endpoint overviews in the risk assessment sections, it is also clearly indicated whether it concerns old or new studies.

A14111B is a suspension concentrate (SC) containing 400 g/L chlorothalonil and 80 g/L azoxystrobin for use as a fungicide in cereals and other speciality crops. A14111B was not the representative formulation in the EU review of chlorothalonil; for further details refer to the confidential dossier of this submission (Document J).

Azoxystrobin which was included into Annex I of Council Directive 91/414/EEC (Commission Directive 1998/47/EC; 7 July 1998) and for which a renewal of this inclusion was voted by SCoFCAH on 9 July 2010 (Commission Directive 2010/55/EU; 20 August 2010). This active substance is an approved active substance under Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011.

Each section of this document provides the agreed EU endpoints and/or proposed amended endpoints (for discussion of amended endpoints, see volume 1).

Where new guidance documents have been introduced since the EU review of chlorothalonil, an updated evaluation of chlorothalonil and A14111B has been included. To adequately assess A14111B to the new guidance documents, it may have been necessary to provide new data, if so these are also included.
The risks from the active substance chlorothalonil and the formulation A14111B are addressed herein. The risk from azoxystrobin is not assessed, except where it is necessary to assess combined toxicity when data with the formulation is not available or not adequate/appropriate.

More detailed information on the composition of A14111B can be found in the confidential dossier of this submission (Document J).

The propsed uses of A14111B are as a fungicide in cereals (spring and winter) and tomatoes. A table of the proposed uses is shown below.

Table 9-1: Use pattern of A14111B (spray application)

Crop	Application	Spray	Number of	Minimum	Maximum individual application	Application

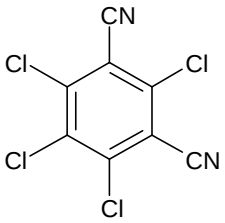
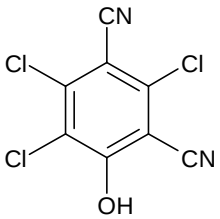
	method	volume (L/ha)	applications	application interval (days)	rate			timing
					L A14111B/ha	g CHTL/ha	g AZT/ha	
Wheat	Spray	100 - 400	2	14 (not before GS 40)	1.875	750	150	BBCH 30-69
Barley								
Tomatoes		500 - 1500	1	-	2.5	1000	200	BBCH 51-89

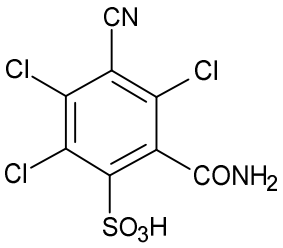
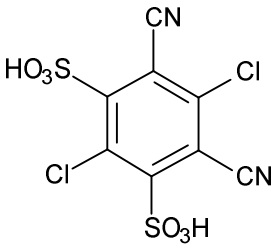
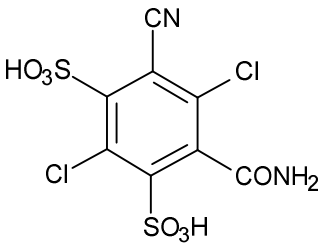
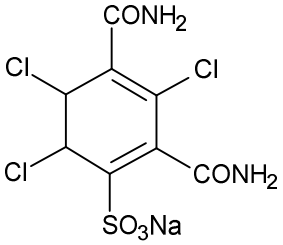
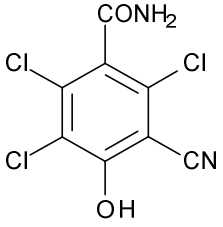
Chlorothalonil is not an isomeric compound. Further consideration of the isomeric composition in the risk assessment is therefore not required.

Consideration of metabolites

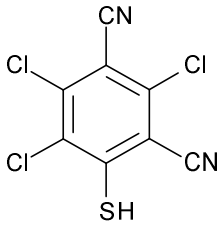
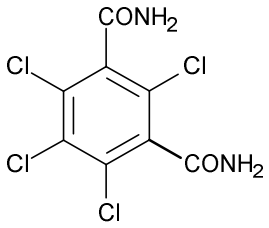
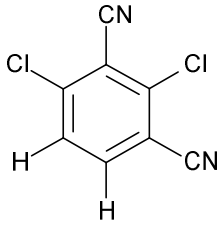
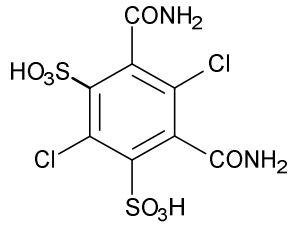
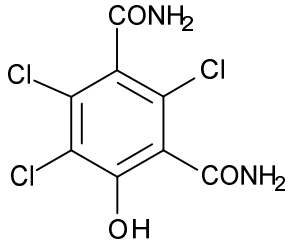
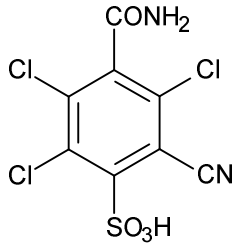
A full list of metabolites and their synonyms was provided by the applicant and is reproduced here:

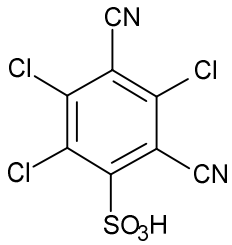
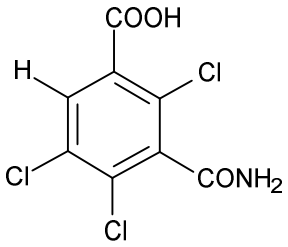
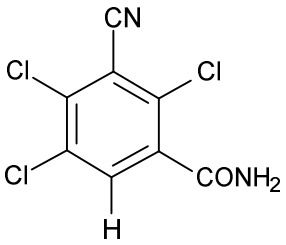
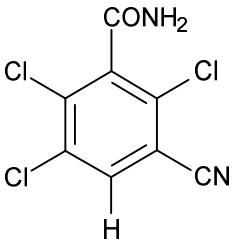
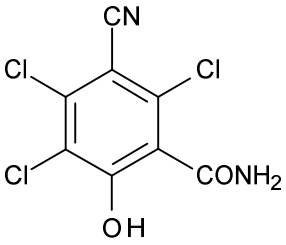
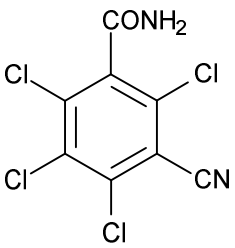
Table 9-2: Metabolites of chlorothalonil

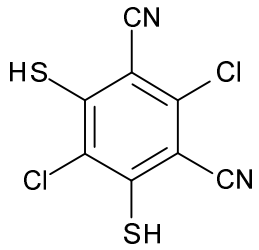
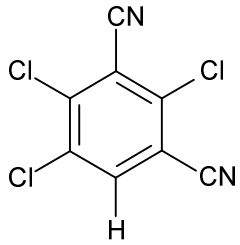
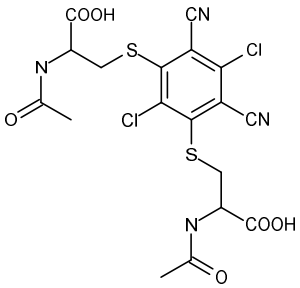
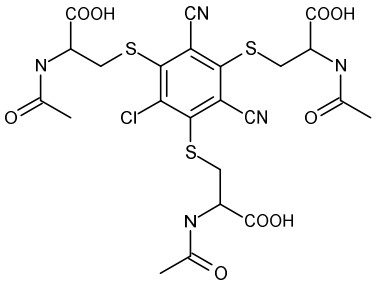
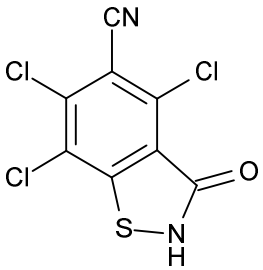
Code Number (Synonyms)	Description	Compound found in:	Structure
Chlorothalonil R044686 SDS 2787 1897-45-6	IUPAC name: 2,4,5,6-tetrachloro-isophtalonitrile	Soil (aerobic, anaerobic, photolysis) Aquatic (water-sediment) Crop (lettuce, tomato, carrot, celery, snap beans, wheat) Rat	
R182281 SDS 3701 R1 Compound 2 C5 28343-61-5 CSCA105253	IUPAC name: 2,5,6-trichloro-4-hydroxyisophthalonitrile	Soil (aerobic, anaerobic, photolysis) Aquatic (hydrolysis) Crop (lettuce, tomato, carrot, wheat, rotated crops) Livestock	

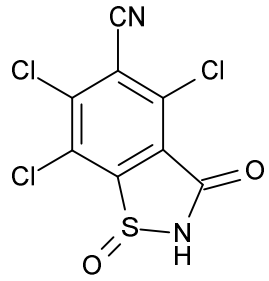
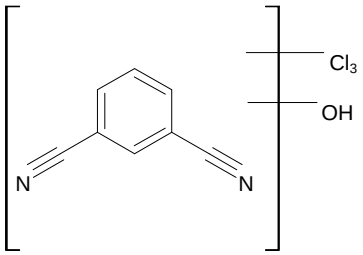
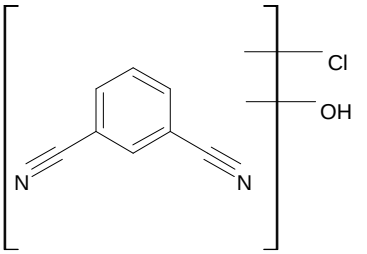
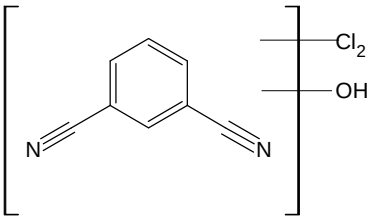
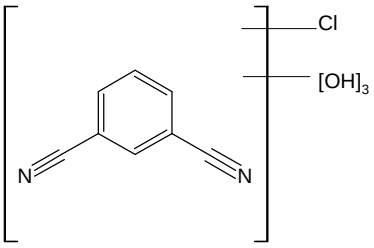
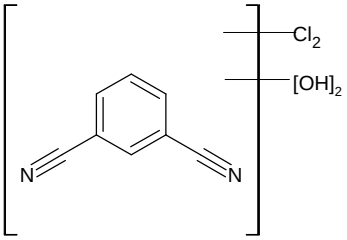
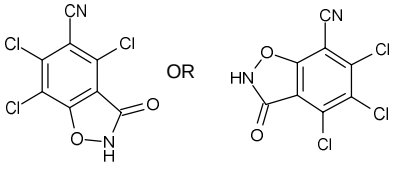
Code Number (Synonyms)	Description	Compound found in:	Structure
		(hen, goat) Rat	
R417888 M12 VIS01 R6 Compound 10 U6 CSCC890840	IUPAC name: 2-amido-3,5,6-trichloro-4-cyanobenzenesulfonic acid	Soil (aerobic, anaerobic) Crop (rotated crops) Rat	
R418503 M13 R8 Compound 11 CSCA654600 SYN548708 (Na salt)[1]	IUPAC name: 2,5 dichloro-4,6 dicyano-benzene-1,3 disulfonic acid	Soil (aerobic) Crop (rotated crops)	
R419492 M8 R15 Compound 12 CSCA655149	IUPAC name: 4-amido-2,5-dichloro-6-cyano benzene-1,3-disulfonic acid	Soil (aerobic) Rat	
R471811 M4 R7 Compound 13 CSCA202566	IUPAC name: sodium 2,4-bis-amido-3,5,6-trichlorobenzenesulfonate	Soil (aerobic) Crop (rotated crops)	
SYN507900 SDS66882 CSCC210323	IUPAC name: 2,4,5-trichloro-3-cyano-6-hydroxy-benzamide	Soil (aerobic, anaerobic)	

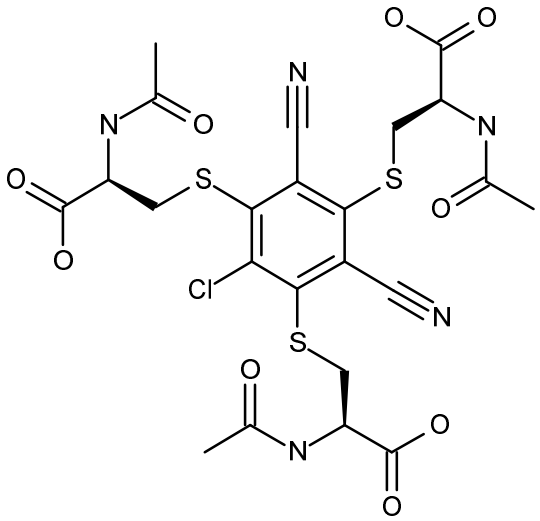
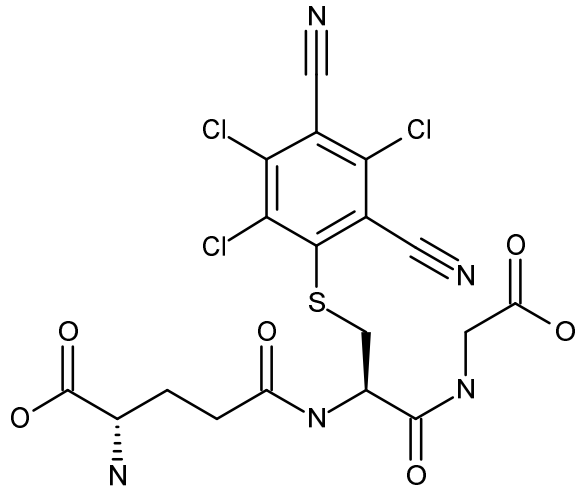
[1] used for gentox testing

Code Number (Synonyms)	**Description**	**Compound found in:**	**Structure**
SYN546671 (C6)	IUPAC name: 2,4,5-trichloro-6-mercaptoisophtalonitrile	Aquatic (Water-sediment)	
SYN546872 VIS-02 R3 CSCR120264	IUPAC name: 2,4,5,6-tetrachlorobenzene-1,3-dicarboxamide	Aquatic (Hydrolysis)	
SYN546934 R613910 MM196	IUPAC name: Dichloro-1,3-dicyanobenzene	Aquatic (aqueous photolysis)	
SYN548008 M3 CSCY735822	IUPAC name: 4,6-dicarbamoyl-2,5-dichloro-benzene-1,3-disulfonic acid	Lysimeter	
SYN548580 M2 R12 CSDB870985	IUPAC name: 2,4,5-trichloro-6-hydroxy-benzene-1,3-dicarboxamide	Lysimeter	
SYN548581 M11 CSDB870988	IUPAC name: 2,3,6-trichloro-5-cyano-4-sulfanyl-benzamide	Lysimeter	

Code Number (Synonyms)	**Description**	**Compound found in:**	**Structure**
R611553 R4 Compound 9 CSCC926922	IUPAC: 3,5,6-trichloro-2,4-dicyano-benzenesulfonic acid	Crop (rotated crops)	
R611965 M5 SDS 46851 R14 Compound 4	IUPAC name: 3-amido-2,4,5-trichlorobenzoic acid	Soil (aerobic, anaerobic) Crop (snap beans, rotated crops) Rat	
R611966 SDS 47523 Compound 5	IUPAC name: 2,4,5-trichloro-3-cyano benzamide	Soil (aerobic, anaerobic) Rat	
R611967 SDS 47524 Compound 6	IUPAC name: 2,5,6-trichloro-3-cyano benzamide	Soil (aerobic)	
R611968 M9 SDS 47525 R5	IUPAC name: 2,4,5-trichloro-3-cyano-6-hydroxybenzamide	Lysimeter Crop (rotated crops)	
R613636 M14 SDS 19221 R2 Compound 3 CSCC548417	IUPAC name: 2,4,5,6-tetrachloro-3-cyanobenzamide	Soil (aerobic, anaerobic) Aquatic (hydrolysis) Crop (rotated crops)	

Code Number (Synonyms)	Description	Compound found in:	Structure
R613800 C15	2,5-dichloro-4,6-bis(sulfanyl)benzene-1,3-dicarbonitrile	Crop (rotated crops)	
R613801 SDS 005473 MM230 C-1 CSAA509968 AGR359-025 CNIL/14	IUPAC name: 2,4,5-trichlorobenzene-1,3-dicarbonitrile	Aquatic (Aquatic photolysis, Water-sediment)	
R613823	IUPAC name: 2-acetamido-3-[3-(2-acetamido-3-hydroxy-3-oxo-propyl)sulfanyl-2,5-dichloro-4,6-dicyano-phenyl]sulfanyl-propanoic acid	Rat	
R613825	IUPAC name: 2-acetamido-3-[3,5-bis[(2-acetamido-3-hydroxy-3-oxo-propyl) sulfanyl]-4-chloro-2,6-dicyano-phenyl] sulfanyl-propanoic acid	Rat	
R613841 SDS67042 Compound 8 CSCC548553	IUPAC name: 4,6,7-trichloro-3-oxo-1,2-benzothiazole-5-carbonitrile	Aquatic (Water-sediment)	

Code Number (Synonyms)	**Description**	**Compound found in:**	**Structure**
R613842 SDS-67042-sulfoxide CSCC548554	IUPAC name: 4,6,7-trichloro-1,3-dioxo-1,2-benzothiazole-5-carbonitrile	Aquatic (water-sediment)	
R950107 Posulated R182281 isomer*		Aquatic (Hydrolysis)	
PD1	Chloro-hydroxybenzene-1,3-dicarbonitrile*	Aquatic (aqueous photolysis)	
PD2	Dichloro-hydroxybenzene-1,3-dicarbonitrile*	Aquatic (aqueous photolysis)	
PD3	Chloro-trihydroxybenzene-1,3-dicarbonitrile*	Aquatic (aqueous photolysis)	
PD4	Dichloro-dihydroxybenzene-1,3-dicarbonitrile*	Aquatic (aqueous photolysis)	
PD5	Trichloro-oxo-dihydro-benzoxazole-carbonitrile	Aquatic (aqueous photolysis)	

Code Number (Synonyms)	Description	Compound found in:	Structure
SYN546677	(2R)-2-acetamido-3-[3,5-bis[[(2R)-2-acetamido-3-hydroxy-3-oxo-propyl]sulfanyl]-4-chloro-2,6-dicyano-phenyl]sulfanyl-propanoic acid	Soil (aerobic)	
SYN546673	(2S)-2-amino-5-[[(1R)-2-(carboxymethylamino)-2-oxo-1-[(2,3,5-trichloro-4,6-dicyano-phenyl)sulfanylmethyl]ethyl]amino]-5-oxo-pentanoic acid	Soil (aerobic)	

The relevance of these metabolites in the environment has been considered in the environmental fate and behaviour section (CA.8). The metabolites which require ecotoxicological assessment (i.e. to which non-target organisms could be exposed) according to the EFSA Guidance Documents are given below.

Table 9-3: Ecotoxicologically potentially relevant metabolites of chlorothalonil

Compartment	Ecotoxicologically relevant metabolites
Soil	R182281 (SDS-3701), R417888 (VIS-01), R418503, R419492, R471811, SYN507900 (SDS-66882), R611965 (SDS-46851),

Compartment	Ecotoxicologically relevant metabolites
	R611966 (SDS-47523), R611967 (SDS-47524) and R613636 (SDS-19221).
Surface water	R182281 (=SDS 3701), R611965 (=SDS 46851), R417888, R613841, R613842, R613801
Plants*	R182281 (=SDS 3701)

* According to the residues assessment/definition

B.9.1 Effects on birds and other terrestrial vertebrates

Assessment of acute mixture toxicity

According to 'EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)' combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.

For the assessment of acute effects (mortality), a surrogate LD_{50} can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD50 for a mixture of active substances with known toxicity assuming dose additivity:

$$\mathrm{LD}_{50}\ (\mathrm{mix}) = \left(\sum_i \frac{\mathrm{X}(a.s._i)}{\mathrm{LD}_{50}(a.s._i)} \right)^{-1}$$

where:

X (a.s.i) = fraction of active substance (i) in the formulation mixture

LD_{50} (a.s.i) = acute toxicity for the active substance (i)

The LD_{50} of the mixture is summarized in Table 9.1.1-2 below.

Table 9.1.1-2: Acute LD_{50} for the mixture of active substances

Test substance	Concentration of active substance in formulation A14111B (g/L)	Fraction of active substance in the formulation mixture[a]	Acute toxicity endpoint (mg/kg bw)	Fraction of active substance/LD_{50} for the active substance	LD_{50} mix (mg/kg bw)
Birds					
Azoxystrobin	80	0.167	> 2000	<0.0000835	**> 2000**
Chlorothalonil	400	0.833	> 2000	<0.000417	
Total	480	1	-	<0.000500	
Mammals					
Azoxystrobin	80	0.167	>5000	<0.000033	**>5000**
Chlorothalonil	400	0.833	>5000	<0.000167	
Total	480	1	-	<0.000200	

[a] Concentration of an active substance in the formulation, divided by the total concentration of all active substances in the formulation.

The value of > 2000 mg/kg bw will be used in the acute risk assessment of the formulation for birds. For mammals, an acute oral toxicity study is available (see K-CP, 7.1.1/01, Kuhn (2004)), which indicates an acute oral LD_{50} of A14111B of 3045 mg/kg in female rats. This value will be used in the acute risk assessment for mammals.

B.9.1.1 Effects on birds

There were no studies with the formulated product submitted. According to the data requirements (284/2013/EC), "The acute oral toxicity of the plant protection product shall be investigated if toxicity cannot be predicted on the basis of the data for the active substance, or where results from mammalian testing give evidence of higher toxicity of the plant protection product compared to the active substance, unless the applicant shows that it is not likely that birds are exposed to the plant protection product itself."

According to the aquatic and mammalian toxicology testing the formulation is less toxic to rats and fish.

The RMS has looked at the composition of A14111B, which contains 33% w/w chlorothalonil and 6% w/w azoxystrobin. There are 3 co-components classified for mammalian toxicity, and they are all present at less than 5% of the formulated product.

Considering the above, the RMS does not consider further testing in birds necessary. The acute risk to birds is considered to be covered by the risk assessment with the active substances.

B.9.1.1.1 Higher tier data on birds

Report:	K-CP 10.1.1.2/01. Miersch, C & Hahne, J., 2014
Title:	Generic Field Study on the Foraging Behaviour of Yellow Wagtails in Tomato Fields in Italy.
Document No:	Tier3 solutions GmbH, Kolberger Strasse 61-63, 51381 Leverkusen, Germany. Report No. B12063-3 Syngenta File Number NA_13441 (Data owner: Bayer Crop Science, Syngenta access)
Guidelines:	No official test guideline(s) available at present. The study was conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009)
GLP	Yes

Executive summary

The foraging behaviour of yellow wagtail in row vegetables, represented by tomatoes, was studied in Italy. Observations demonstrated that approximately 30 % of feeding was on foliage and 70 % of feeding on the ground.

Study Design and Methods:

Experimental dates: 5th June 2013 - 14th July 2013

In the current study specific emphasis was put on the feeding behaviour of the insectivorous Yellow Wagtail in row vegetable fields, as represented by tomato fields. The aim was to quantify the

proportion to which Yellow Wagtails forage on the ground vs. in the foliage as both strata are differently exposed to crop protection products during application. This information can be collected specifically for a species and crop type and can be generically used for the refinement on different crop protection products.

The study was conducted in the Province of Lodi/Lombardy and in the Province of Piacenza/Emilia-Romagna, Italy. Both sites are typical areas for tomato growing. Study fields were selected to be representative for commercially managed tomato fields, the presence of the focal species and the suitability for the study conduct (i.e. availability of different crops and habitat types as well as accessibility of fields).

Altogether 23 tomato fields were scanned for the presence of Yellow Wagtails, however, successful feeding observations were made only on 6 fields. Foraging observations were done either in the morning or in the evening, in order to match high bird activity. If possible, single birds were observed for their feeding behaviour until at least 20 feeding events (defined as 'successful' pecks) were noted where the strata from which the food was taken (ground or foliage) could be specified. A single bird observation was limited by either identifying at least 20 feeding events, by the foraging duration or by the duration the bird was visible.

Results:

Altogether 23 tomato fields were scanned for the presence of Yellow Wagtails. Useful observations of the feeding behaviour of single individuals (in terms of quantification on feeding behavior), however, could only be made on 6 fields. Here, altogether 29 different feeding observations could be made, comprising 133 successful pecks with known stratum (87 from the ground, 46 from plants) and 23 pecks from unknown stratum.

Table CP 10.1.1.2-01: Summary of feeding observations for Yellow Wagtails feeding in tomatoes

Feeding observation No.	No. of pecks from ground	No. of pecks from foliage	Total no. of pecks	% pecks from ground	% pecks from foliage
1	1	5	6	16.7	83.3
2	1	0	1	100.0	0.0
3	5	1	6	83.3	16.7
4	2	0	2	100.0	0.0
5	3	0	3	100.0	0.0
6	5	0	5	100.0	0.0
7	0	2	2	0.0	100.0
8	1	2	3	33.3	66.7
9	2	0	2	100.0	0.0
10	0	1	1	0.0	100.0
11	1	7	8	12.5	87.5
12	0	4	4	0.0	100.0
13	1	0	1	100	0.0
14	3	0	3	100	0.0
15	2	0	2	100	0.0

16	2	2	4	50.0	50.0
17	2	0	2	100	0.0
18	1	1	2	50.0	50.0
19	6	3	9	66.7	33.3
20	20	13	33	60.6	39.4
21	1	0	1	100.0	0.0
22	1	0	1	100.0	0.0
23	6	0	6	100.0	0.0
24	3	0	3	100.0	0.0
25	1	0	1	100.0	0.0
26	11	3	14	78.6	21.4
27	2	0	2	100.0	0.0
28	4	0	4	100.0	0.0
29	0	2	2	0.0	100.0
Ssum of pecks	**87**	**46**	**133**	**Mean over birds**	
Percentage of pecks	**65.4%**	**34.6%**		**70.7%**	**29.3%**

Considering the foraging proportion per feeding observation in calculation, the average proportion of pecks from foliage was 29.3% and the average proportion of pecks from the ground was 70.7% as shown in the table above.

Conclusion:

Overall, a ratio of approximately 30 % foliage feeding and 70 % ground feeding of Yellow wagtails in row vegetable fields such as tomato fields could be concluded.

(Miersch C, Hahne J, 2014)

Study Comments: 10.1.2_01	The author concludes that the ratio between foliage feeding and ground feeding is approximately 3:7. Observations were only done by eye not by radiotracking. However, visibility of birds foraging in and between plants is less than visibility of birds foraging on the soil. Birds were seen to fly into the crop and disappeared. It is not clear if they were foraging on the floor or not. In Table A1 (from the report) a number of comments confirm this statement: not visible when covered by plants/disappearing between the tomato plants/spending time inside the rows, action not visible. Furthermore, it is questionable if results from feedings with a limited number of pecks can be used. E.g. some observations consist of only 1 peck and are then counted as 100%. Birds that are only observed pecking once have a much higher weight on the ratio then birds with several pecks. It would have been better to leave out all birds with for example 4 or less observed pecks and count total foliar and ground pecks for the remaining birds. This would be based on only 8 birds (see figure 4) but the outcome is more reliable.
Agreed Endpoint(s): 10.1.2_01	The PD refinement cannot be used for risk assessment.

Miersch, C & Hahne, J	2014	Generic Field Study on the Foraging Behaviour of Yellow Wagtails in Tomato Fields in Italy.	10.1.1.2_01

Reliability: 3	
General information	
Is a guideline method or modified guideline used?*	No official test guideline(s) available at present. The study was conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009)
Is the test performed under GLP conditions?*	Yes
If applicable, are validity criteria fulfilled (e.g. control survival, growth, etc.)?	n.a.
Are appropriate controls performed (e.g. solvent control, negative and/or positive control)?	n.a.
* these criteria are of minor importance for study reliability, but may support study evaluation	
Test compound	
Is the test substance clearly identified with name or CAS-number? Are test results reported for the appropriate compound?	n.a.
Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	n.a.
If a formulation is used or if impurities are present: do other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	n.a.
Test organism	
Are the organisms well described (e.g. scientific name, weight, length, growth, age/life stage, strain/clone, gender if appropriate)?	n.a.
Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?	n.a.
Exposure conditions	
Is the experimental system appropriate for the test substance, taking into account its physicochemical characteristics?	n.a.
Is the experimental system appropriate for the test organism? Have conditions been stable during the test?	n.a.
If appropriate, were exposure concentrations below the limit of water solubility (taking the use of a solvent into account)? If a solvent is used, is the solvent within the appropriate range and is a solvent control included?	n.a.
Is a correct spacing between exposure concentrations applied?	n.a.
Is the exposure duration defined?	n.a.
If necessary, are chemical analyses adequate to verify concentrations of the test substance over the duration of the study?	n.a.
Where applicable, is the biomass loading of the organisms in the test system within the appropriate range?	n.a.
Statistical Design and Biological Response	
Is a sufficient number of replicates used? Is a	n.a.

B.9.1.2 Effects on terrestrial vertebrates other than birds

There were no studies with the formulated product submitted which are only applicable for the terrestrial vertebrates risk assessment. For mammalian studies with the formulated product A14111B, please see section CP 6.1.1. The study showed that the acute toxicity of the formulation is in the range of the toxicity studies with the active substance(s).

B.9.1.2.1 Higher tier data on mammals

Report:	K-CP 10.1.2/01. Sainz-Elipe S, Haehne J, 2014
Title:	Generic field study on the attractiveness of tomato fields for Savi's pine voles in Italy
Document No:	Tier3 Solutions GmbH, Kolberger Strasse 61-63, 5381 Leverkusen, Germany. Report Number M489745-01-1 Syngenta File Number NA_13506 (Data owned by BCS, Syngenta access)
Guidelines:	No official test guideline(s) available at present. The study was conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009).
GLP	No
Previous evaluation	Submitted for the renewal (new study)
RMS conclusion	Acceptable for use in risk assessment.

Executive summary

The aim of this generic study was to determine the attractiveness of commercially managed tomato fields for Savi's pine voles (*Microtus savii*). The study was conducted in the Province of Lodi/Lombardy as well as in the Province of Piacenza/ Emilia-Romagna, Italy. Live trapping of small mammals was conducted both in-crop and off-field to determine which small mammals use tomato fields. Savi's pine vole was the most frequently trapped small mammal in off-field habitats with captures per trap night more than twice that of the next most frequently captured species, the wood mouse (*Apodemus sylvaticus*). However, Savi's pine voles were never caught in-field and here the wood mouse was the most frequently trapped small mammal.

Study Design and Methods

Experimental dates: 5 June 2013 to 14 July 2013.

The study was conducted in the Province of Lodi/Lombardy and in the Province of Piacenza/ Emilia-Romagna, Italy on tomato fields. Altogether 14 study fields were selected to be representative for commercially managed tomato fields, the potential presence of the focal species and the suitability for the study conduct (e.g. accessibility of fields).

Live trappings of small mammals with individual marking and subsequent recaptures were conducted in tomato fields and in the adjacent off-crop habitats in the period between early June until mid of July, corresponding to growth stages of tomato plants between BBCH 13 and BBCH 77. For regular

trappings at each study site live traps were distributed in a regular trapping grid with 53 traps. Two rows of each trapping grid, comprising 15 traps, were set in the adjacent off-crop area, whilst the other 38 traps were distributed within the tomato fields. Additional sets of 20 traps each were installed and frequently moved between fields in order to increase trapping success. Animals which were trapped for the first time were individually marked by means of transponders. For each captured individual the following information was taken: date, location (position in study field and trap identity; i.e. number of trap and row), species, ID of PIT (if applied), first capture or recapture, sex, reproductive state and body weight.

Results

During 8,591 trap nights altogether 395 captures of small mammals were made. Six species were trapped during the study period: the black rat (*Rattus rattus*), the house mouse (*Mus musculus*), the wood mouse (*Apodemus sylvaticus*), the Savi's pine vole (*Microtus savii*), the Eurasian harvest mouse (*Micromys minutus*) and the bicoloured white-toothed shrew (*Crocidura leucodon*). The wood mouse was the most trapped species (105 individuals) followed by *M. savii* (92 individuals). The highest trapping success, however, was obtained for Savi's pine vole in traps set up in the adjacent off-crop habitats (7.54 captures per 100 trap nights; see table below). This species was trapped in 13 of 14 study sites and not a single *M. savii* was recorded inside the tomato fields in the course of the study i.e. all *M. savii* were trapped in the off-crop habitats adjacent to the tomato fields.

Table 1: Small mammal trapping in and adjacent to tomato fields

Species	Standardised trapping success [captures/100 trap nights]		
	Tomato field	Adjacent off-crop habitats	TOTAL
Microtus savii	0.00	7.54	1.80
Apodemus sylvaticus	2.16	3.12	2.39
Mus musculus	0.09	0.34	0.15
Rattus rattus	0.00	0.05	0.01
Micromys minutus	0.12	0.10	0.12
Crocidura leucodon	0.00	0.44	0.10
Sorex sp.	0.00	0.10	0.02

Conclusion

In the current study it could be shown that Savi's pine vole (*Microtus savii*) was absent on tomato fields. Savi's pine voles and wood mouse (*Apodemus sylvaticus*) were frequently trapped in the off-crop habitats adjacent to tomato fields (capture rates of 7.54 and 3.12 per 100 trap nights respectively), but not a single *M. savii* was trapped inside of tomato field irrespective of developing growth stages of tomato plants (BBCH stages). In the tomato fields, the capture rate of *A.sylvaticus* was the highest with 2.16 per 100 trap nights.

(Sainz-Elipe & Haehne, 2014)

Study Comments: 10.1.2_01	*Micromys minutus* was found in this study although this is not a species that was expected to occur in southern Italy. This species may be a more

	suitable species to use as a focal species since it is mostly granivorous- but also consumes plant matter and insects.
Agreed Endpoint(s): 10.1.2_01	The most common mammal in tomato fields in Italy was the wood mouse (*Apodemus sylvaticus*) with a capture rate of 2.16 per 100 trap nights.

Sainz-Elipe S, Haehne J.	2014	Generic field study on the attractiveness of tomato fields for Savi's pine voles in Italy	10.1.2_01

General information	
Is a guideline method or modified guideline used?*	No official test guideline(s) available at present. The study was conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009).
Is the test performed under GLP conditions?*	Yes
If applicable, are validity criteria fulfilled (e.g. control survival, growth, etc.)?	n.a.
Are appropriate controls performed (e.g. solvent control, negative and/or positive control)?	n.a.
* these criteria are of minor importance for study reliability, but may support study evaluation	
Test compound	
Is the test substance clearly identified with name or CAS-number? Are test results reported for the appropriate compound?	n.a.
Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	n.a.
If a formulation is used or if impurities are present: do other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	n.a.
Test organism	
Are the organisms well described (e.g. scientific name, weight, length, growth, age/life stage, strain/clone, gender if appropriate)?	n.a.
Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?	n.a.
Exposure conditions	
Is the experimental system appropriate for the test substance, taking into account its physicochemical characteristics?	n.a.
Is the experimental system appropriate for the test organism? Have conditions been stable during the test?	n.a.
If appropriate, were exposure concentrations below the limit of water solubility (taking the use of a solvent into account)? If a solvent is used, is the solvent within the appropriate range and is a solvent control included?	n.a.
Is a correct spacing between exposure concentrations applied?	n.a.
Is the exposure duration defined?	n.a.
If necessary, are chemical analyses adequate to verify concentrations of the test substance over the duration of the study?	n.a.
Where applicable, is the biomass loading of the organisms in the test system within the appropriate range?	n.a.
Statistical Design and Biological Response	
Is a sufficient number of replicates used? Is a sufficient number of organisms per replicate	n.a.

B.9.1.3 Residues of chlorthalonil in relevant food items

Study K-CP 10.1.1/04 Schmidt 2009

Report:	Schmidt, T., 2009
Title:	Determination of Residue Levels of chlorothalonil on/in non-Target Arthropods in the Laboratory
Document No:	Harlan Laboratories Ltd., Itingen, Switzerland, Report No. C34632 Syngenta file No. R044686_11258
Guidelines:	Guidance document of the Scientific Panel on Plant Protection Products and their Residues for the Risk Assessment for Birds and Mammals under Council 91/414/EEC Directive (2007). Recommendations on Arthropod residue Field Studies to Refine Food residues in Higher Tiered Bird and Mammal Risk Assessments (2007).
GLP	Yes

Executive summary:

The purpose of this study was to determine the initial residue levels of the active ingredient chlorothalonil after one application with the formulation Chlorothalonil 500 g/L SC on/in representatives of arthropods, as surrogates of food sources for birds, under laboratory conditions on soil planted with barley.

Adults and nymphs of the phytophagous brown cricket *Acheta domestica* and larvae of the saprophagous black beetle species *Zophobas* spec. were used. They were confined in plastic containers and exposed to the test item. The substrate in the plastic containers was bare soil planted with barley. Three replicates per species and sampling date were used. The test item Chlorothalonil 500 g/L SC (a formulation nominally containing 500 g a.s./L) was applied using a laboratory track sprayer. The application rate was 2000 mL Chlorothalonil 500 g/L SC per ha, equivalent to 1000 g a.s./ha. The test organisms were sampled at several sampling intervals up to 14 days after application and were deep-frozen prior to residue analysis. Homogenized samples of test organisms were extracted with sodium sulphate and toluene. Chlorothalonil was quantified by GC-ECD. The limit of quantification (LOQ) of the analytical method in the two insect species was 0.05 mg/kg fresh weight for chlorothalonil. The limit of detection (LOD) for chlorothalonil was found to be 0.02 mg/kg fresh weight.

The mean residue level of chlorothalonil in/on *Zophobas* larvae was 1.161 mg/kg fresh weight shortly after application and decreased to 0.045 mg/kg fresh weight 7 days after application. Afterwards, the residue levels varied between 0.093 and 0.062 mg/kg fresh weight up to 14 days after application. The DT_{50}-value for chlorothalonil in *Zophobas* larvae was calculated to be 14.5 hours (corresponding to 0.6 days).

The mean residue level of chlorothalonil in/on crickets was 5.05 mg/kg fresh weight shortly after application and increased to 6.140 mg/kg fresh weigh 4 hours after application. Afterwards, the residue levels decreased to 0.223 mg/kg fresh weight up to 10 days after application. No values were

obtained for the test organisms exposed for 14 days since mortality was very high. The DT_{50}-value for chlorothalonil in crickets was calculated to be 22.6 hours (corresponding to 0.94 days).

I. MATERIALS AND METHODS

A. MATERIALS

1.Test Material:	Chlorothalonil 500 g/L SC
Description:	Liquid
Lot/Batch #:	G246
Purity:	Nominal: chlorothalonil 500 g/L
	Measured: chlorothalonil 485.1 g/L
Stability of test compound	May 2009 (expiry date)
2. Vehicle and/or positive control	Deionized water
3. Test animals	
Species:	*Acheta domestica* (brown cricket)
	Zophobas sp. (black beetle)
Strain	Not relevant
Age:	Adults and nymphs
	Larvae
Weight at dosing:	Not relevant
Source:	Zoohandlung Schaub - Liestal (Switzerland)
Acclimation period	Not relevant
Diet:	Sliced carrots
Water:	Test containers were moistened daily, if necessary.
Housing:	Exposure units consisting in plastic containers (bottom approx. 38 × 22 cm, top approx. 42 × 25 cm, height approx. 18 cm). A layer of soil (depth approx. 2-5 cm, "Anzuchterde", *i.e.* a type of soil which is recommended for growing seedlings) was filled into the test containers as test substrate. Barley was planted at a density of 45 seeds in three rows. At the day of application, the planted barley seedlings were reduced to a density of 36 plants per container (corresponding to 430 plants/m^2). The plants had reached 2nd leaf growth stage (*i.e.* GS 12 on the BBCH scale, BBA 2001) and had a uniform height of approximately 10 cm. The test organisms were introduced into the test containers directly before application. Afterwards, the containers with the crickets were covered with a coarsely meshed gaze with a pore size of approximately 10 mm to prevent the test organisms from

	escaping.
4. Environmental conditions	
Temperature:	18.7 – 19.3 °C
Humidity:	64 – 76 %
Air changes:	Not relevant
Photoperiod:	16:8 hour light: dark; intensity: 4480 – 7000 lux
B. STUDY DESIGN AND METHODS	
1. In life dates	9 - 23 February 2009
2. Experimental treatment	The following rate was tested: 1.0 kg chlorothalonil/ ha (=2.0 L product/ha, corresponding to 5.0 mL product/L, based on an application volume of 400 L/ha). Prior to the start of the study, the application solution for the application rate was prepared by dispersing 10 mL of test item in 2000 mL deionized water. Then, an adequate volume of this application solution was sprayed onto the surface of the barley plants of each test container by means of track sprayer (Spray Lab from Schachtner, Germany). The sprayer was calibrated to deliver a target of 4 ± 0.4 mg spray solution/cm^2, corresponding to 400 L/ha, by weighing the amount of water delivered. Before application, spray patterns was checked visually for uniformity. The control consisted of two replicates with each 15 crickets and 29 *Zophobas* larvae, which were sampled before test item application.
3. Observations	The test organisms were sampled shortly after application and 4, 24, 48, 96, 168, 240 and 336 hours after treatment (HAT) and deep-frozen prior to residue analysis.
4. Statistics	Best-fit model FOMC; Best-fit model SFO.
5. Study deviations	None.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean residue level of chlorothalonil in/on *Zophobas* larvae was 1.161 mg/kg fresh weight shortly after application, and decreased to 0.045 mg/kg fresh weight 7 days after application. Afterwards, the residue levels varied between 0.093 and 0.062 mg/kg fresh weight up to 14 days after application. The mean residue levels and the survival of the test organisms are detailed in the table below.

Table 1:Mean Residue Levels of chlorothalonil (mg/kg/fresh weight) and survival for *Zophobas larvae*

Time after application		Mean number of test organisms per replicate				% survival		chlorothalonil (mg/kg fresh weight)	
Day	Hours	introduced	dead	not found	surviving	mean	s.d.	mean	s.d.
-0	-0	15	0.0	0.0	29.0	100.0	0.0	< 0.05 (n.d.)	---
0	0	29	0.0	0.0	29.0	100.0	0.0	1.161	0.318
0	4	15	0.0	1.3	27.7	95.4	2.0	0.757	0.087
1	24	15	0.0	4.3	24.7	85.1	8.7	0.529	0.187
2	48	15	0.7	1.3	27.0	93.1	9.1	0.360	0.211
4	96	15	0.7	4.0	24.3	83.9	13.9	0.102	0.034
7	168	15	0.7	0.3	28.0	96.6	3.4	0.045	0.045
10	240	15	0.3	5.0	23.7	81.6	7.2	0.093	0.010
14	336	15	2.3	5.3	21.3	73.6	8.0	0.062	0.015

n.d.: Not determined.

The mean residue level of chlorothalonil in/on crickets was 5.050 mg/kg fresh weight shortly after application and increased to 6.140 mg/kg fresh weigh 4 hours after application. Afterwards, the residue levels decreased to 0.223 mg/kg fresh weight up to 10 days after application. From Day 10 on, replicates with low survival (*i.e.* < 40 %) were excluded from further calculations since residue levels of dead test organisms varied unpredictably. No values were obtained for the test organisms exposed for 14 days since mortality was very high. The mean residue levels and the survival of the test organisms are detailed in the table below.

Table 2: Mean Residue Levels of chlorothalonil (mg/kg/fresh weight) and survival for crickets

Time after application		Mean number of test organisms per replicate				% survival		chlorothalonil (mg/kg fresh weight)	
Day	Hour	introduced	dead	not found	surviving	mean	s.d.	mean	s.d.
-0	-0	15	0.0	0.0	15.0	100.0	0.0	< 0.05 (n.d.)	---
0	0	15	0.0	0.0	15.0	100.0	0.0	5.050	2.033
0	4	15	0.0	1.0	14.0	93.3	6.7	6.140	5.663
1	24	15	0.0	1.0	14.0	93.3	11.5	1.654	0.214
2	48	15	3.3	1.3	10.3	68.9	26.9	1.787	0.789
4	96	15	5.0	2.3	7.7	51.1	7.7	0.868	0.442
7	168 a	15	4.0	2.0	9.0	60.0	n.d.	0.171	n.d.
10	240 a	15	3.0	4.0	8.0	53.3	n.d.	0.223	n.d.
14	336 a	15	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.d.: Not determined.

a: Replicates with survival < 40 % were excluded from the calculations since residue levels in/on dead test organisms can change in an unpredictable way.

n.a.: Not applicable.

III. CONCLUSIONS

The DT_{50} value for *Zophobas* larvae was calculated to be 14.5 hours (corresponding to 0.6 days). The DT_{50} value for crickets was calculated to be 22.6 hours (corresponding to 0.94 days).

The risk can be refined considering a more realistic PT and PT values, the portion of an animal's daily diet obtained in habitat treated with pesticides.

Schmidt, T., 2009

Study Comments: 10.1.1_03	Analytical method is considered acceptable. Variation in chlorothalonil concentrations in crickets is high, especially after 4 hours. Reported concentrations were 0.135, 6.899 and 11.385 mg/kg fwt. the lowest concentration is lower than any other sample, while the highest concentration is about 2 times the initial concentration. Variation at 96 hours was also high and on days 7 and 10 only one replicate was used for the analysis. The DT_{50} values were recalculated using CAKE (computer assisted kinetic evaluation, version 3.3) and SFO resulting in a DT50 of 1.16 days ($r^2$0.86). The DT_{50} value for chlorothalonil in crickets was calculated to be 0.954 days (r^2 0.35). The DT_{50} value for chlorothalonil in crickets is not considered reliable since r^2 is very low (0.35).
Agreed Endpoint(s): 10.1.1_03	The DT50 for chlorothalonil in *Zophobas larvae* can be used for risk refinement.

Schmidt, T.	2009	Determination of Residue Levels of chlorothalonil on/in non-Target Arthropods in the Laboratory	10.1.1-03

Reliability: 2	
General information	
Is a guideline method or modified guideline used?*	Yes
Is the test performed under GLP conditions?*	Yes
If applicable, are validity criteria fulfilled (e.g. control survival, growth, etc.)?	n.a.
Are appropriate controls performed (e.g. solvent control, negative and/or positive control)?	yes
* these criteria are of minor importance for study reliability, but may support study evaluation	
Test compound	
Is the test substance clearly identified with name or CAS-number? Are test results reported for the appropriate compound?	Yes Yes
Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	Yes
If a formulation is used or if impurities are present: do other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	n.a. (active substance)
Test organism	
Are the organisms well described (e.g. scientific name, weight, length, growth, age/life stage, strain/clone, gender if appropriate)?	No size related observations only life-stages reported
Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?	Yes No pre-exposure to the test substance
Exposure conditions	
Is the experimental system appropriate for the test substance, taking into account its physicochemical characteristics?	Yes
Is the experimental system appropriate for the test organism? Have conditions been stable during the test?	Yes Yes
If appropriate, were exposure concentrations below the limit of water solubility (taking the use	-

B.9.1.4 Residues of SDS-3701 in relevant food items

To address the risk to birds and mammals from the metabolite SDS-3701, the notifier refers to a report (Edwards, 2001) generated for the Annex I inclusion and used in Addendum 14 to the DAR. A summary of this report was not presented in the Addendum and is therefore presented below.

Study CP 10.1.1/01 Edwards P. 2001 SDS-3701 plant residues

Report:	Edwards P (2001). Chlorothalonil: Risk Assessment for Birds and Wild Mammals from Agricultural use in Europe. (Syngenta File No. R44686/0664).

Guidelines

None cited

GLP: No

Executive Summary

ECCO 110 asked Syngenta to prepare a risk assessment to address the risk to birds and mammals from long-term exposure to chlorothalonil and its metabolite SDS-3701 (R182281). In addition the Rapporteur Member State (RMS) was asked to check the data on mammalian toxicology and establish the appropriate toxicity endpoint for the long-term risk assessment. This document focuses on the residue analysis.

Initial residue estimates were made according to Luttik's recommendations in the draft EU Expert group document. Typically crops receive multiple applications of chlorothalonil, so 21-day maximum moving Time Weighted Average residues on potential food items have been used. These estimates are based on initial 50th percentile residues from Fletcher et al (1994) and Fischer and Bowers (1997) and DT_{50} values estimated from an extensive crop residue database. Residues of SDS 3701 on potential food items have been estimated directly from this crop residue database. Residues have been presented as 50th and 90th percentiles for use in risk assessment.

For SDS-3701, 50th and 90th percentile residues were estimated from a large database of field crop residue studies conducted in North America. Risk to birds and wild mammals using typical 'worst case scenarios' as defined in the draft EU Expert Group proposals was low for all uses of chlorothalonil.

The entire SDS-3701 residue database was examined to see if SDS-3701 accumulates in time (DALA) and if residues accumulate on vegetation or increase in proportion to the application rate. If this is not the case then we can be confident that 50th percentile residues in the database represent robust values for long-term risk assessment.

The database comprises a mixture of crops (with different surface area to mass characteristics), application rates and sampling intervals after the last application (DALA - days after the last application).

Syngenta believe that 90th and 50th percentile residues of the metabolite, SDS-3701, can be used for exposure estimates in risk assessment. Dry plants and peanut hulls are a reasonable worst-case surrogate for insects.

Study Design and Methods

Residues of chlorothalonil will be highest on potential wildlife food items immediately after an application. SDS-3701 is primarily a soil metabolite and not a major plant metabolite. Chlorothalonil and SDS-3701 residues are dynamic so the ratio will change with time. Thus, the use of SDS-3701: chlorothalonil ratios is not an appropriate method to estimate exposure levels of SDS-3701 from estimated levels of chlorothalonil.

A large field crop residue database is available for both chlorothalonil and SDS-3701. This large field crop residue database was considered to be more relevant and robust than the limited metabolism studies conducted in the greenhouse. This extensive residue data has therefore been used to confirm this dynamic relationship and estimate the 50th and 90th percentile concentrations of SDS-3701 for risk assessment. In adition the data have been used to estimate DT50 values

The residue data for SDS-3701 is compiled from several different studies in the USA and therefore reflects variability from different crop application scenarios and conditions in the field. However, the trend of these actual field data is expected to follow the theoretical distributions with residues of SDS-3701 being significantly less than residues of chlorothalonil and SDS-3701 reaching a peak after application and then decreasing to low or undetectable concentrations. This pattern is best observed from examination of the largest residue database for fruit.

Results and Discussion

Residues of chlorothalonil declined quickly in response to days after last application (DALA).

Residues of SDS-3701 were typically 2 to 3 orders of magnitude less than chlorothalonil. Residues of SDS-3701 did not accumulate with increasing DALA and appear to decline almost as quickly as chlorothalonil, demonstrating rapid formation and dissipation of SDS-3701. SDS-3701 residues did not appear to increase in response to the application rate and there was little difference in the response when comparing residues with either the total or final application rate.

Dry plants, peanut hulls and similar vegetation do not constitute wildlife food items. However, dry plants may be considered a worst-case surrogate for insect food items because of their proximity to the soil surface. Residues of SDS-3701 on dry plant were typically one order of magnitude less than chlorothalonil. Residues of SDS-3701 did not accumulate with increasing DALA but did appear to decline more slowly than on grass, green vegetables and fruit. SDS-3701 residues appear to increase in response to the total application rate. This relationship was not apparent for the final application rate.

Table 1: Use of the Residue Database in Appendix 3 for estimation of residue percentiles in risk assessment.

Crop Types described in the Residues Database	Crop Groups described in the Residue Database	Crop Groups used in Appendix 3	Wildlife Food Groups used in Table 17	No's samples for percentile estimates
Turf clippings	Short grass	Short grass	Short grass	93
Corn fodder	Long grass	Green vegetables	Long grass	25
Cabbage Sprouts	Broadleaf		Leafy crops	11
Cauliflower Broccoli	Vegetable flowers		NA	11
Onions, Asparagus Celery	Vegetables on ground		NA	
Snap beans Pigeon peas (seeds in pods)	Vegetable green beans		NA	
Blackberry, Blueberry Boysenberry, Cranberry Grape, Raspberry Strawberry, Tart cherry Sweet cherry	Small Fruit	Fruit	Fruit	360
Apricot, Japanese plum Nectarine, Passion fruit Peach, Prune, Tomato	Medium Fruit			
Cucumber, Mango, Orange, Squash, Papaya, Watermelon	Large Fruit			
Grass seed	Small seed		Seed	60
Corn grain, Wheat grain	Large seed			
Dry edible beans Lentils, Pigeon peas Soybean	Pulses		NA	
Almonds, Filberts Pecan, Pistachios	Nut kernels above ground		NA	
Peanut nutmeats	Peanuts		NA	
Peanut hulls	Peanut hulls	Peanut Hulls	NA	
Dry edible bean plant Peanut hay and forage Wheat straw	Dry plants	Dry plants	= Insects	56

Grass seed straw				

Table 2: 50th and 90th percentile estimates of chlorothalonil and SDS-3701 using the crop residue database.

Vegetation type	Total applic'n rate (kg/ha) (lb/ha = 0.4536 kg/ha)*	Measured chlorothalonil residues (mg/kg fresh weight)		Measured SDS-3701 residues (mg/kg fresh weight)	
		50th percentile	90th percentile	50th percentile	90th percentile
Short grass	5.5-49 (= 2.5-22)	250	1700	1.8	5.2
Long grass	10.5 (= 4.8)	4.4	23.3	0.05	0.17
Leafy crops	9-15.2 (= 4.1-6.9)	0.23	5.9	0.005	0.03
Vegetable flowers	4.7-12.9 (= 2.1-5.9)	1.8	5.8	0.013	0.09
Fruit	2.0-53 (= 0.9-24)	0.43	5.1	0.015	0.025
Seed (single sample of grass seed)	1.5-10.5 (4.5) (= 0.7-4.8)	0.015	0.015 (43.5)	0.015	0.015 (0.49)
Dry vegetation = insect	1.5-9 (= 0.7-4.1)	0.47	13	0.18	0.86

*The table and the body text of the document referred to by the notifier mention application rates in kg a.i./ha, but according to the appendices the application rates mentioned in the table are in lb a.s./ha. It is not known which is correct. To ensure a worst case approach, Ctgb recalculated the values to kg a.s./ha.

Measured residues of chlorothalonil and SDS-3701 were substantially higher on short grass than on other vegetation types, reflecting the very high application rates (from turf use in US) and low mass of the grass.

Estimated DT_{50} values of chlorothalonil are presented in Table 3.

Table 3: Estimated DT_{50} values for chlorothalonil

Food Source	DT_{50} (days)
Short grass	2.1
Long grass and leafy crops	11.4
Seeds & Grain	6.2
Fruit	14
Insects	6.2[1]

[1] Assumed to be equal to grain because of similar surface area

Table 4: Long term exposure of SDS-3701 to birds and mammals

Crop category	Crop stage	Typical feeding guild	Food source for estimating residues	C mg/kg diet (50th percentile measured)
Cereal/ grassland	Early	H mammal	Short grass	1.8
		H bird	Short grass	1.8
	Late	I mammal	Insects	0.18
		I bird	Insects	0.18
Vegetables	Early	H mammal	Leafy crops	0.05
		H bird	Leafy crops	0.05
	Late	I mammal	Insects	0.18
		I bird	Insects	0.18
Orchard	Early /Late	H mammal	Short grass[1]	0.9
		I bird	Insects	0.18

[1] 50% interception applied to the C (50th percentile)

Validity Criteria

Not relevant.

Conclusions

In conclusion, Syngenta believe that 90th and 50th percentile residues of the metabolite, SDS-3701, can be used for exposure estimates in risk assessment. Dry plants and peanut hulls are a reasonable worst-case surrogate for insects. Table 1 lists all the crops sampled for understanding the relationship between chlorothalonil and SDS-3701 and used for the estimation of residue percentiles (Table 2).

Remarks RMS

The overall quality of the studies used cannot be evaluated, as these data are not available to the RMS. However, taking into consideration the large amount of data evaluated, the RMS agrees that this data is indicative of the residue dynamics and levels of the plant metabolite SDS-3701 in plant material. This information may potentially be used in the risk assessment for birds and mammals but the uncertainties should be carefully considered, including: climatic and geographic conditions are unknown, details of the study (sampling and sample storage) are unknown, the method of calculation of DT_{50}s is unknown. The conclusion that the residues levels are independent of dose rate cannot be fully confirmed, as the pattern of residues levels of SDS-3701 closely followed that of chlorothalonil. In the largest group (fruit) higher residues seemed generally to be seen at higher application rates, but this relationship could not be confirmed in the other plant groups for either chlorothalonil or the metabolite SDS-3701. A full discussion of these uncertainties and the manner in which the RMS will use this information in the risk assessment may be found in CP Section 9.2.1, Metabolites.

Study CP 10.1.1/02 King and Ballee (1987)

Report:	K-CP 10.1.1/02 King C. and Ballee D.L. (1987), Residues of 4-hydroxy-2,5,6-trichloro-isophthalonitrile (SDS-3701) on turf clippings. Department of Environmental Sciences, Riscerca, Inc. 7528 Auburn road P.O. Box 1000 Painesville, Ohio 44077. Syngenta Report Number 1391-86-0078-CR-001. (Syngenta File No: R44686/2258).

Guidelines

In accordance with protocol number 1391-86-0078-CR-000 and protocol amendment number 1391-86-0078-CR- 000-001

GLP: yes

Executive Summary

In this study, turf clippings were analyzed for SDS-3701 on two sets of samples; one set was collected from the 19th practice green at Deer Lake Golf Course Geneva, Ohio and another set of samples was collected from the 19th practice green from Quail hollow Golf Course, Painesville, Ohio. Each collection contained samples before DACONIL 2787 Flowable Fungicide application and continuous sampling with each mowing after treatment.

A maximum mean level of 0.77 mg SDS-3701/kg sample was detected on Deer Lake samples taken 1 day following a field application. Deer Lake samples taken at intervals longer than 1 day following an application contained < 0.50 mg SDS-3701/kg sample (the non-detect level). A maximum mean level of 6.69 mg SDS-3701/kg sample was detected on Quail hollow samples. The levels of SDS-3701 residues on Quail hollow samples were significantly higher than the levels on Deer Lake samples and remained at the same level longer before starting to decline. This probably is due to a combination of higher rate of DACONIL 2787 Flowable Fungicide applied in this study and to the use of DACONIL 2787 Flowable Fungicide in the normal greens maintenance program earlier in the season at Quail hollow. The earlier applications of fungicide probably also account for the detection of SDS-3701 in the "pre-spray" samples from quail Hollow. There were no previous sprays of DACONIL 2787 Flowable Fungicide prior to the conduct of this study at Deer Lake Golf Course. At both golf courses loss of SDS-3701 on grass clippings was observed over a period of time with subsequent mowings.

I. MATERIALS AND METHODS

A. MATERIALS

A1. Test Materials

Test Material	DACONIL 2787 Flowable Fungicide
Purity	40.6% chlorothalonil
Batch number	BBSLSRU

A2. Test Facilities

The test plots were located in Painesville, Ohio and Geneva, Ohio.

The assay of turf clippings was conducted at the Department of Environmental Sciences, Ricerca, Inc., 7528 Auburn Road, Painesville, Ohio 44077

B. STUDY DESIGN AND METHODS

B1. Application and Sampling

The application of DACONIL 2787 Flowable Fungicide and sampling of turf clippings was conducted under the direction of the Commercial Development Staff of the Fermenta Plant Protection Company. The test plots were located in Painesville, Ohio and Geneva, Ohio. Applications were made using DACONIL 2787 Flowable Fungicide, a dispersible suspension containing a minimum of 40.4% chlorothalonil.

The application rate : 3.85 oz/1000 sq. ft. at Deer Lake on 14, 20 and 26 August 1985. 7.51 oz/1000 sq. ft. at Quail Hollow on 17 and 24 September 1985. Based on the US product label of Daconil 2787 Flowable Fungicide (https://www3.epa.gov/pesticides/chem_search/ppls/050534-00009-20081010.pdf), 3 fl. oz./1000 sq ft equals 4.16 lb a.i./acre. With 1 lb/acre = 1.12 kg/ha, the reported application rates of 3.85 and 7.51 oz/1000 sq ft for Deer Lake and Quail Hollow equal 5.98 and 11.7 kg a.i/ha, respectively.

Previous applications: none at Deer Lake; 5 oz/1000 sq. ft. (7.8 kg a.i./ha) on 21 August 1985 and 4 oz/1000 sq.ft (6.2 kg a.i./ha) on 10 September 1985 at Quail Hollow.

The spray solution was applied using commercial tractor mounted turf sprayers. At each sampling date, turf clippings were collected during mowing from each of the treated 19th greens. These samples were taken to the Environmental Science Laboratories where they were maintained under frozen conditions until assay.

Diseases Controlled [a]	Application Interval (days)	Pre-Disease Rates[a]			Post-Disease Rates[a]		
		fl. oz. product/ 1000 sq ft	pints product/ acre	lbs a.i./ acre	fl. oz. product/ 1000 sq ft	pints product/ acre	lbs a.i./ acre
Dollar Spot	7 to 10	1.5[b] to 3	4[b] to 8	2.1[b] to 4.16	-	-	-
	7 to 21	3 to 5	8 to 14	4.16 to 7.3	-	-	-
	14	-	-	-	6 to 8	16 to 22	8.32 to 11.3

B2. Analytical Phase

Untreated turf clippings were amended in the blender cup prior to extraction by the addition of a standard solution of SDS-3701. The turf clippings were amended at a minimum of five concentrations within the range of 0.50 to 30.0 mg SDS-3701/kg sample. The amended samples were processed through the described analytical procedure to evaluate the validity of the assay procedure. The assay procedure was validated prior to initiation of sample assay. Residues of SDS-3701 were extracted from the turf clippings and selectively partitioned into an organic solvent. The residue SDS-3701 was derivatized to its methyl ether and cleaned up by column chromatography prior to quantitation.

II. RESULTS AND DISCUSSION

Validation of Assay Procedure

Turf was amended in a range of 0.50 to 30.0 mg SDS-3701/kg turf and resulted in a % recovery ranging from 79% to 112% with a mean of 94%. These data indicate that the analytical procedure is valid for the determination of SDS-3701 on turf clippings.

Assay Values

The assay values for SDS-3701 on turf clippings from untreated plots and plots treated with DACONIL 2787 Flowable Fungicide are presented in Ttables 10.1.1-7 and 10.1.1-8 In these tables, the non-detect (N.D.) level is defined as <0.50 mg SDS-3701/kg sample which is the lowest level of demonstrated recovery of SDS-3701 from turf clippings through the analytical procedure.

TABLE 10.1.1-7 : RESIDUES (mg/kg sample) OF 4-HYDROXY-2,5,6-TRICHLOROISOPHTHALONITRILE (SDS-3701) ON TURF CLIPPINGS FOLLOWING APPLICATIONS OF DACONIL 2787 FLOWABLE FUNGICIDE (BATCH BBSLSRU) AT 5.98 kg.a.i./ha FROM A TEST CONDUCTED AT DEER LAKE GOLF COURSE, GENEVA, OHIO

Application type	Application Dates	Mowing Date	PMI[1] Days	SDS-3701 mg/kg (between brackets: RUD[2] -)
Untreated – Pre-Spray	NA	8/14/85	NA[4]	ND
				ND
				ND
				Mean ND
Treated - 1 Application	8/14/85	8/15/85	1	0.83 (0.14)
				0.71 (0.12)
				0.76 (0.13)
				Mean 0.77
Treated – 1 Application	8/14/85	8/17/85	3	ND[3]
				ND
				ND
				Mean ND
Treated – 1 Application	8/14/85	8/19/85	5	ND
				D
				ND
				Mean ND
Treated – 2 Applications	8/14/85	8/21/85	1	ND
	8/20/85			ND
				ND
				Mean ND
Treated – 2 Applications	8/14/85	8/26/85	6	ND[3]
	8/20/85			ND
				ND
				Mean ND
Treated – 3 Applications	8/14/85	8/27/85	1	0.59 (0.099)
	8/20/85			0.57 (0.095)
	8/26/85			0.60 (0.10)
				Mean 0.59 (0.099)

1:PMI - Pre-mowing Interval

2: RUD: Residue per unit dose, calculated as the measured residue of SDS-3701 in mg/kg grass divided by the application rate of 5.98 kg a.i./ha

3: ND - <0.5 mg SDS-370/kg sample

4: NA – Not applicable

TABLE 10.1.1-8 : RESIDUES (PPH) OF 4-HYDROXY-2,5,6-TRICHLOROISOPHTHALONITRILE (SDS-3701) ON TURF CLIPPINGS FOLLOWING APPLICATIONS OF DACONIL 2787 FLOYABLE FUNGICIDE (BATCH BBSLSRU) AT 11.7 kg a.i./ha FROM A TEST CONDUCTED AT QUAIL HOLLOW GOLF COURSE, PAINESVILLE, OHIO

Application type	Application Dates	Mowing Date	PMI[1] Days	SDS-3701 mg/kg (between brackets: RUD[3] - d)
Pre-Spray		9-17-85	NA[2]	1.08 (0.092)
				1.07 (0.091)
				1.09 (0.093)
				Mean 1.08 (0.092)
Treated – 1 Application	9-17-85	9-18-85	1	5.68 (0.49)
				5.76 (0.49)
				5.58 (0.48)
				Mean 5.67 (0.49)
Treated – 1 Application	9-17-85	9-19-85	2	6.01 (0.51)
				5.91 (0.51)
				5.89 (0.50)
				Mean 5.94 (0.51)
Treated – 1 Application	9-17-85	9-20-85	3	5.93 (0.51)
				5.91 (0.51)
				5.88 (0.50)
				Mean 5.91 (0.51)
Treated – 1 Application	9-17-85	9-21-85	4	6.56 (0.56)
				6.7 (0.57)
				6.81 (0.58)
				Mean 6.69 (0.57)
Treated – 1 Application	9-17-85	9-22-85	5	5.11 (0.44)
				5.19 (0.44)
				5.57 (0.48)
				Mean 5.29 (0.45)
Treated – 1 Application	9-17-85	9-23-85	6[4]	1.65 (0.14)
				1.69 (0.14)
				1.70 (0.15)

Application type	**Application Dates**	**Mowing Date**	**PMI[1] Days**	**SDS-3701 mg/kg (between brackets: RUD[3] - d)**
				Mean 1.68 (0.14)
Treated – 1 Application	9-17-85	9-24-85	7	ND[3]
				ND
				ND
				Mean ND
Treated – 2 Applications	9-17-85	9-25-85	1	6.2 (0.53)
	9-24-85			6.38 (0.55)
				6.52 (0.56)
				Mean 6.37 (0.54)
Treated – 2 Applications	9-17-85	9-26-85	2[4]	4.7 (0.40)
	9-24-85			4.91 (0.42)
				4.84 (0.41)
				Mean 4.82 (0.41)
Treated – 2 Applications	9-17-85	9-27-85	3	0.62 (0.053)
	9-24-85			0.76 (0.065)
				0.67 (0.057)
				Mean 0.68 (0.058)
Treated – 2 Applications	9-17-85	9-28-85	4	0.71 (0.060)
	9-24-85			0.6 (0.051)
				0.61 (0.052)
				Mean 0.64 (0.055)

1: PMI - Pre-mowing Interval

2: NA – Not applicable

3: RUD: Residue per unit dose, calculated as the measured residue of SDS-3701 in mg/kg grass divided by the application rate of 11.7 kg a.i./ha

4: 2 & 6 - Greens were cut lower on this day for tournament play.

TABLE 10.1.1-9: DEER LAKE MEAN RESIDUE LEVEL - SUMMARY

Application type	PMI days[1]	Days after first application (Only relevant for 2 and 3 application types.)	SDS-3701 ppm (RUD[2] - mg/kg ai applied)
1 applications	1		0.77 (0.13)
	3		ND[3]
	5		ND
2 applications	1	7	ND
	6	12	ND

3 applications	1	13	0.59 (0.10)

1: PMI - Pre-mowing Interval (= days after last application)

2: RUD: Residue per unit dose, , calculated as the measured residue of SDS-3701 in mg/kg grass divided by the application rate of 5.98 kg a.i./ha

3: ND - <0.50 mg SDS-3701/kg sample

TABLE 10.1.1-10 : QUAIL HOLLOW MEAN RESIDUE LEVEL - SUMMARY

Application type	PMI days[1]	Days after first application (Only relevant for 2 and 3 application types.)	SDS-3701 mg/kg sample (RUD[2] - mg/kg ai applied)
1 application	1		5.67 (0.48)
	2		5.94 (0.51)
	3		5.91 (0.51)
	4		6.69 (0.57)
	5		5.29 (0.45)
	6		1.68 (0.14)
	7		ND[3]
2 applications	1	8	6.37 (0.54)
	2	9	4.82 (0.41)
	3	7	0.68 (0.058)
	4	11	0.64 (0.055)

1: PMI - Pre-mowing Interval

2: RUD: Residue per unit dose, calculated as the measured residue of SDS-3701 in mg/kg grass divided by the application rate of 11.7 kg a.i./ha

3: ND - <0.50 mg SDS-3701/kg sample

III. CONCLUSIONS

In this study, turf clippings were analyzed for SDS-3701 on two sets of samples; one set was collected from the 19th practice green at Deer Lake Golf Course Geneva, Ohio and another set of samples was collected from the 19th practice green from Quail hollow Golf Course, Painesville, Ohio. Each collection contained samples before DACONIL 2787 Flowable Fungicide application and continuous sampling with each mowing after treatment.

A maximum mean level of 0.77 mg SDS-3701/kg sample was detected on Deer Lake samples taken 1 day following a field application. Deer Lake samples taken at intervals longer than 1 day following an application contained < 0.50 mg SDS-3701/kg sample (the non-detect level). A maximum mean level of 6.69 mg SDS-3701/kg sample was detected on Quail hollow samples. The levels of 505-3701 residues on Quail hollow samples were significantly higher than the levels on Deer Lake samples and remained at the same level longer before starting to decline. This probably is due to a combination of higher rate of DACONIL 2787 Flowable Fungicide applied in this study and to the use of DACONIL

2787 Flowable Fungicide in the normal greens maintenance program earlier in the season at Quail hollow. The earlier applications of fungicide probably also account for the detection of SDS-3701 in the "pre-spray" samples from Quail Hollow. There were no previous sprays of DACONIL 2787 Flowable Fungicide prior to the conduct of this study at Deer Lake Golf Course. At both golf courses loss of SDS-3701 on grass clippings was observed over a period of time with subsequent mowings.
King C. and Ballee D.L. (1987)

Study Comments: KCP 10.1.1/02	At Deer Lake, the average residue of SDS-3701 one day after the 1st application was 0.77 mg/kg. This is consistent with residues of 0.59 mg/kg one day after the 3rd application. However, no residues were detected after the 2nd application. No explanation is given for this. Higher amounts of residues in the Quail Hollow samples are explained by the applicant by the fact that two applications were performed earlier in the season in the normal maintance program. Higher amounts of residues were also explained by higher applications dosages used in the Quail Hollow than at the Deer Lake location. However, from the difference between pre-spray samples (1.08 mg/kg) and samples taken one day after the first spray (5.67 mg/kg), it follows that the 1st application resulted in residues of 4.3 mg/kg. This is 5 to 7 times higher than the residues at Deer Lake, while the application rate at Quail Hollow was only 2 times higher. Because verification of dosage and application rate was not performed, it is not clear if inhomogeneous application may be a cause of the differences. Unintended inclusion of soil particles in the extraction may also be a factor. Mowing was performed at different time intervals after application. It is not clear from the report, however, if different plots were used for the respective time intervals, or that plots that were sampled later on had also been mowed in between. As the experiments were performed on greens that are probably in full operation, a standard mowing regime is suspected of less than one week. In the application type with two applications the mowings were performed 7 to 13 days after the first application. For a green in full operation this would not be possible. This sustains the suspicion that mowing have been performed in between. Wheather conditions are not reported and it is not clear if rainfall has caused residue decline by washing off. The US label gives a concentration of 4.16 lb a.i./gallon (498 g/L) for a 40.4% formulation. This leads to a density of 1.23 g/mL, which is consistent with the MSDS (1.24 g/mL; http://www.plantproducts.com/ca/images/daconil_15724_en_msds.pdf).

	With 1 US fl oz = 0.007815 gallon, and 1 sq ft = 2.30 x 10^{-5} acre, the application rate of 3 fl oz / 1000 sq ft is 4.25 lb a.i./acre, and not 4.16 as indicated in the label. Using the density of the product of 1.24 g/mL, the reported a.i. content in the study (40.6%), the conversion factor from US fl oz to L (0.029574) and from sq ft to m^2 (0.092903), the application rate of 3.85 oz/sq ft = 6.2 kg a.i./ha and the application rate of 7.51 oz/sq ft = 12.0 kg a.i./ha, which is almost similar to the figures reported by the applicant (5.98 and 11.7 kg a.i./ha). However, it is not fully clear if the calculation of the application rate is correct, since the application rate is not specifically given as fluid ounces, but only as 'oz'. Overall, the study report lacks a clear description of the application and sampling methods and the conditions during the study. This hampers the evaluation of the results and the relevance for the present risk assessment is unclear. For this reason the results are unreliable. However, the results can be regarded as underestimations of the residue levels when in between, not reported but suspected mowings and removal of the clippings have decreased the level of the residues. If this was the case the results may approach the actual residue levels.
Agreed Endpoint(s): KCP 10.1.1/02	The results cannot be used for risk assessment because the of several uncertainties in the study set-up and reporting. Results can only be used as supporting information.

King and Ballee	1987	Residues of 4-hydroxy-2,5,6-trichloro-on turf clippings	CP 10.1.1/02

Reliability	
General information	
Is a guideline method or modified guideline used?*	n
Is the test performed under GLP conditions?*	n
If applicable, are validity criteria fulfilled (e.g. control survival, growth, etc.)?	n
Are appropriate controls performed (e.g. solvent control, negative and/or positive control)?	y
* these criteria are of minor importance for study reliability, but may support study evaluation	
Test compound	
Is the test substance clearly identified with name or CAS-number? Are test results reported for the appropriate compound?	y
Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	y
If a formulation is used or if impurities are present: do other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	Na y
Test organism	
Are the organisms well described (e.g. scientific name, weight, length, growth, age/life stage, strain/clone, gender if appropriate)?	na
Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?	na
Exposure conditions	
Is the experimental system appropriate for the test substance, taking into account its physicochemical characteristics?	y
Is the experimental system appropriate for the test organism? Have conditions been stable during the test?	Y Conditions not reported
If appropriate, were exposure concentrations below the limit of water solubility (taking the use	Y

Study CP 10.1.1/03 Cassidy, Cillon and Ballee (1991)

Report:	K-CP 10.1.1/03. Cassidy P.S., Dillon K.A. and Ballee D.L. (1991), Residues of Tetrachloroisophthalonitrile (chlorothalonil, SDS-2787), SDS-3701, SDS-46851, HCB, and PCBN on turf clippings. Riscerca, Inc. Department of Environmental Sciences, 7528 Auburn road P.O. Box 1000 Painesville, Ohio 44077. Syngenta Report Number 1391-86-0078-CR-001. (Syngenta File No: R44686/2299).

This report looks at residues of chlorothalonil, SDS-3701, SDS-46851, HCB, and PCBN on turf clippings. Ony the data for chlorothalonil and SDS-3701 are presented below.

Guidelines

In accordance with protocol number 1642-90-0323-CR-000

GLP: yes

Executive Summary

In this study, turf clippings from 4 greens located at Quail Hollow Golf Course, Painesville, Ohio were analyzed for residues of chlorothalonil (SDS-2787) and SDS-3701. The samples were taken before application of DACONIL 2787 Flowable Fungicide and continuous sampling with each mowing after treatment (days 1 to 7).

A total of 3 treatments were applied to 4 greens. As this golf course uses chlorothalonil for turf maintenance of its greens, previous applications of DACONIL 2787 Flowable Fungicide had been made at the test sites thus accounting the presence of all compounds in pretreated grass clippings. After treatment, declines were seen in the residues of chlorothalonil.

A statistical calculation of the data indicates that the decline rate in residue level is 46% per day for SDS-2787. The predicted cumulative declines within 7 days were 99% for chlorothalonil. Residues of SDS-3701 were fairly constant throughout the study except for days one to three after each application when SDS-3701 showed a noticeable decline. The data demonstrates that the metabolites will not build up in turf with repeated applications of DACONIL 2787 Flowable Fungicide.

I. MATERIALS AND METHODS

A. MATERIALS

A1. Test Materials

Test Material	Chlorothalonil (SDS-2787)
Purity	99.6%
Batch number	SDS-2787-1501
Test Material	SDS-3701
Purity	99.5%
Batch number	SDS-3701-0201

A2. Test Facilities

Quail Hollow Golf Course, Painesville, Ohio.
The assay of turf clippings was conducted at the Department of Environmental Sciences, Ricerca, Inc., 7528 Auburn Road, Painesville, Ohio 44077. Assays were conducted from 23rd August 1990 to 30th November 1990.
Three applications of Daconil 2787 Flowable were made at seven day intervals in four replicates (greens). Two samples of grass clippings were taken from each greens daily over a twenty-one day period.

Previous applications: The summary mentions that previous applications were made at this location. The previous study mentions 5 oz/1000 sq. ft. on 21 August /1985 and 4 oz/1000 sq.ft on 10 September 1985 at Quail Hollow.
7.51 oz/1000 sq. ft. at Quail Hollow on 17 and 24 September 1985 (study design of previous study). Applications in between 1985 and 1991 are not documented.

B. STUDY DESIGN AND METHODS

B1. Processing phase

Locally obtained untreated turf clippings were amended in the blender jars prior to extraction by the addition of individual standard solutions of chlorothalonil and SDS-3701.
The turf clippings were amended within range of 0.05 mg/kg to 2500 mg/kg for chlorothalonil and within range of 0.03 to 15 mg/kg for SDS-3701. The amended samples were processed through the described analytical procedure to evaluate the validity of the assay procedure. In some instances when large amounts of chlorothalonil were used for spiking a concurrent sample was fortified with the same amount of chlorothalonil to determine SDS-3701 levels associated with chlorothalonil.

B2. Analytical Phase

Chlorothalonil and its metabolite SDS-3701 were extracted from the sample and selectively partitioned into an organic solvent. The residues of chlorothalonil were separated by column chromatography prior to quantitation by electron capture gas chromatography. The residues of SDS-3701 were derivatized to the methyl ether cleaned up and separated by column chromatography prior to quantitation.

II. RESULTS AND DISCUSSION

Validation of Assay Procedure

The validation was untreated turf clippings amended with chlorothalonil and SDS-3701 at respective levels ranging from 0.05 to 2500 mg a.i./kg and 0.03 to 15 mg/kg. The recovery ranged from 77% to 123% with a mean of 102% and from 60% to 124% with a mean of 88% respectively. These data indicate that the analytical procedure is valid for the determination of chlorothalonil and SDS-3701 on turf clippings.

Assay Values

Analysis of the turf clippings showed a rapid decline in the amount of residues of chlorothalonil in days following applications. Highest values of chlorothalonil were found at the first post-application interval (PAI), following the first application at an overall mean value of the 4 greens of 2617 ppm. SDS-3701

residues were more consistent throughout the study, with residues declining only between the first and third PAI after each treatment. SDS-3701 was at its maximum level at the first application and first PAI with an average value of 5.24 ppm.

A statistical evaluation of the data was prepared. Statistical modeling of the samples indicates a decline in the residues of chlorothalonil. The rate of decline for chlorothalonil 46% per day. Based on this calculation at 7 days after application the predicted cumulative declines would be 99% of the original amount of chlorothalonil.

Residues of SDS-3701 declined between day 1 and 3, but leveled off at a mean value of 1.45 ppm. This indicates that the SDS-3701 didn't build up on turf with successive applications.

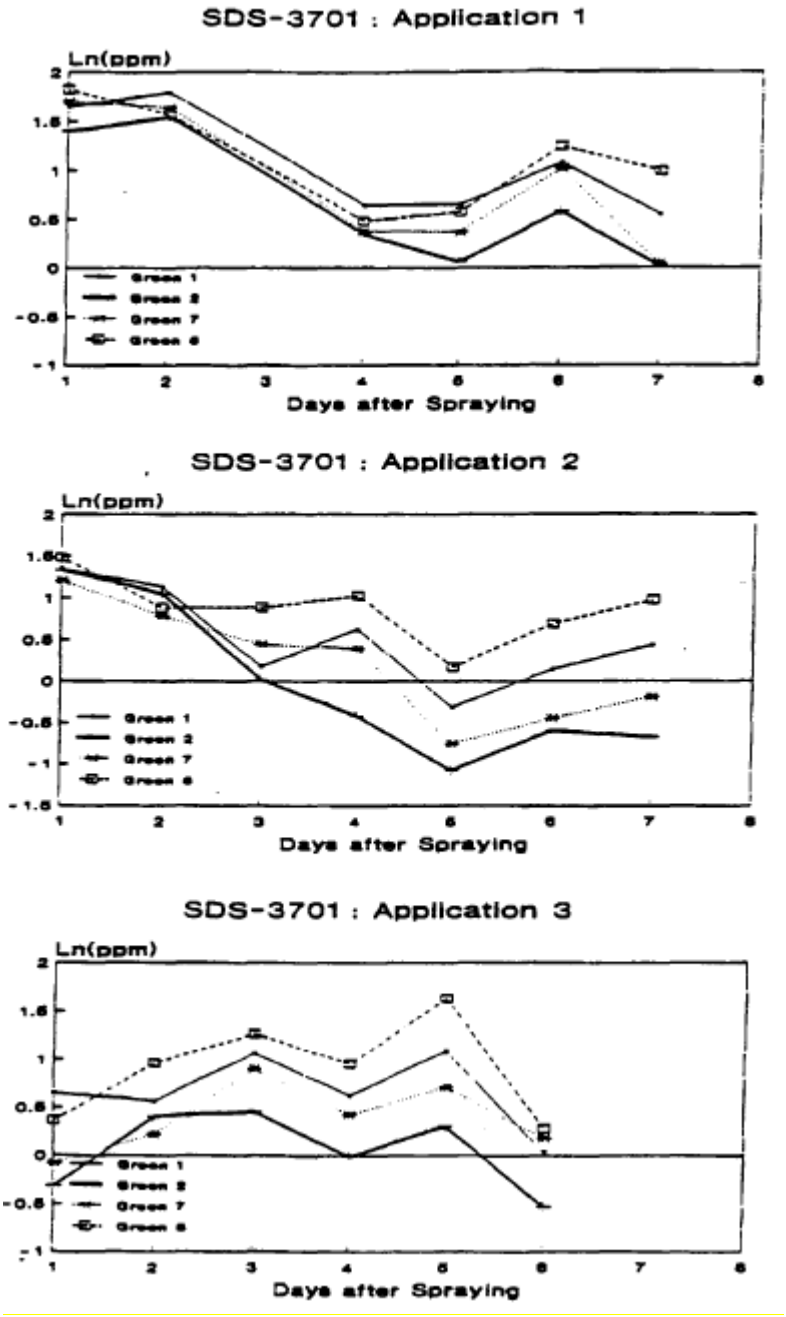
TABLE 10.1.1-11 : Residues (mg ai/kg sample) of chlorothalonil (SDS-2787) and SDS-3701 (means from four greens)

Nr. of applications	PAI[1]	SDS-2787	SDS-3701
0	-1	5.51	0.57
1	1	2617	5.24
1	2	1603	5.18
1	3	482	1.60
1	5	441	1.55
1	6	189	2.74
1	7	82.3	1.62
2	1	685	3.86
2	2	383	2.65
2	3	200	1.55
2	4	136	1.70
2	5	44.4	0.68
2	6	24.2	1.09
2	7	13.1	1.39
3	1	499	1.25
3	2	293	1.78
3	3	224	2.63
3	5	110	1.75
3	6	218	2.87

3	**7**	**196**	**1.04**

1: Post Application Interval (days)

Figure 10.1.1-1. SDS-3701 in turf clippings in three application scenario's



III. CONCLUSIONS

The analytical procedure is valid for the determination SDS-3701in turf clippings as the mean of 88% (SD ±15%) is within the accepted range of 70-120% recovery.

In this study, turf clippings from 4 greens located at Quail Hollow Golf Course, Painesville, Ohio were analyzed for residues of chlorothalonil (SDS-2787) and SDS-3701. The samples were taken before application of DACONIL 2787 Flowable Fungicide and continuous sampling with each mowing after treatment (days 1 to 7).

A total of 3 treatments were applied to 4 greens. As this golf course uses chlorothalonil for turf maintenance of its greens, previous applications of DACONIL 2787 Flowable Fungicide had been made at the test sites thus accounting the presence of all compounds in pretreated grass clippings. After treatment, declines were seen in the residues of chlorothalonil.

A statistical calculation of the data indicates that the decline rate in residue level is 46% per day for SDS-2787. The predicted cumulative declines within 7 days were 99% for chlorothalonil. Residues of SDS-3701 were fairly constant throughout the study except for days one to three after each application when SDS-3701 showed a noticeable decline. The data demonstrates that the metabolites will not build up in turf with repeated applications of DACONIL 2787 Flowable Fungicide.
Cassidy P.S., Dillon K.A. and Ballee D.L. (1991)

Study Comments: KCP 10.1.1/02	Previous applications with chlorothalonil on greens were made in the test locations, giving a background concentration of 0.57 mg SDS-3701/kg sample. It is not described if different plots were used for the respective mowing intervals, or that plots that were sampled later on had also been mowed in between. If the latter is the case, residue decline is expected because young grass is not sprayed. The intended application rate in the present experiment is not given and no information is presented on the actual application rates. No information on weather conditions. Overall, the study is poorly documented. The report lacks a clear description of the application and sampling methods and the conditions during the study. This hampers the evaluation of the results . For instance, it is not explained why residues are so low directly after the third application (1.25 mg SDS-3701/kg sample) in comparison with the residues after the first and second application (5.24 mg and 3.86 SDS-3701/kg sample, respectively) and the relevance for the present risk assessment is unclear. Results can only be used as supporting evidence. Residues of SDS/3701 appear to stay at between 1 – 2 mg per kg sample at day 7 after application. Highest residue level was 5.24 mg SDS-3701/kg sample at day 1 after application.

Agreed Endpoint(s): KCP 10.1.1/02	

Cassidy P.S., Dillon K.A. and Ballee D.L.	1991	Residues of Tetrachloroisophthalonitrile (chlorothalonil, SDS-2787), SDS-3701, SDS-46851, HCB, and PCBN on turf clippings.	K-CP 10.1.1/03.

Reliability	
General information	
Is a guideline method or modified guideline used?*	n
Is the test performed under GLP conditions?*	n
If applicable, are validity criteria fulfilled (e.g. control survival, growth, etc.)?	n
Are appropriate controls performed (e.g. solvent control, negative and/or positive control)?	y
* these criteria are of minor importance for study reliability, but may support study evaluation	
Test compound	
Is the test substance clearly identified with name or CAS-number? Are test results reported for the appropriate compound?	y
Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	y
If a formulation is used or if impurities are present: do other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	Na y
Test organism	
Are the organisms well described (e.g. scientific name, weight, length, growth, age/life stage, strain/clone, gender if appropriate)?	na
Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?	na
Exposure conditions	
Is the experimental system appropriate for the test substance, taking into account its physicochemical characteristics?	y
Is the experimental system appropriate for the test organism? Have conditions been stable during the test?	Y 47 Conditions not reported

B.9.2 Risk assessment for birds and other terrestrial vertebrates

B.9.2.1 Summary of effects on birds and other terrestrial vertebrates

Species	Endpoint	Value [mg a.s./kg bw (/d)]	Old/new dossier	Reference
Birds				
Chlorothalonil				
Acute oral (gavage)				
Anas platyrhynchos (mallard duck)	14d LD_{50}	**> 2000**	Old: DAR 2000 & Review report Chlorothalonil,	Hakin (1992) CA 8.1.1.1/01 (KIIA 8.1.1)
Anas platyrhynchos (mallard duck)	8d LD_{50}	> 4640	Old: DAR 2000 & Review report Chlorothalonil,	Beavers and Fink (1977) CA 8.1.1.1/02 (KIIA 8.1.1)
Coturnix japonica (japanese quail)	14d LD_{50}	**> 2000**	Old: DAR 2000 & Review report Chlorothalonil,	Shults et al. (1987) CA 8.1.1.1/03 (KIIA 8.1.1)
Short-term oral (dietary)				
Colinus virginianus (bobwhite quail)	5d LC_{50}	> 5200 mg/kg diet	Old: DAR 2000 & Review report Chlorothalonil,	Hakin et al. (1992) CA 8.1.1.2/02 (KIIA 8.1.2)
Anas platyrhynchos (mallard duck)	5d LC_{50}	> 5200 mg/kg diet	Old: DAR 2000 & Review report Chlorothalonil,	Hakin et al. (1992) CA 8.1.1.2/01 (KIIA 8.1.2/01)
Colinus virginianus (bobwhite quail)	5d LC_{50}	> 10000 mg/kg diet	Old: DAR 2000 & Review report Chlorothalonil,	Shults et al. (1979) CA 8.1.1.2/03
Anas platyrhynchos (mallard duck)	5d LC_{50}	> 10000 mg/kg diet	Old: DAR 2000 & Review report Chlorothalonil,	Shults et al. (1979) CA 8.1.1.2/04 (KIIA 8.1.2)
Reproduction				
Colinus virginianus (bobwhite quail)	NOAEL	**14.1**	Old: DAR 2000 & Review report Chlorothalonil	Redgrave et al. (1993) CA 8.1.1.3/05 (KIIA 8.1.3)
Anas platyrhynchos (mallard duck)	NOAEL	726	Old: DAR 2000 & Review report Chlorothalonil	Shults et al. (1988) CA 8.1.1.3/01 (KIIA 8.1.3)

Species	Endpoint	Value [mg a.s./kg bw (/d)]	Old/new dossier	Reference
Colinus virginianus (bobwhite quail)	NOAEL	158	Old: DAR 2000 & Review report Chlorothalonil	Shults et al. (1988) CA 8.1.1.3/03
metabolite R182281 (SDS-3701)				
Acute oral (gavage)				
Anas platyrhynchos (mallard duck)	8d LD_{50}	**158** [c]	Old: DAR 2000 & Review report Chlorothalonil,	Killeen et al. (1978) CA 8.1.1.1/04
Short-term oral (dietary)				
Anas platyrhynchos (mallard duck)	5d LC_{50}	2000	Old: DAR 2000 & Review report Chlorothalonil,	Shults et al. (1981) CA 8.1.1.2 / 05 (KIIA 8.1.2)
Colinus virginianus (bobwhite quail)	5d LC_{50}	1780	Old: DAR 2000 & Review report Chlorothalonil,	Shults et al. (1981) CA 8.1.1.2 / 06 (KIIA 8.1.2)
Reproduction				
Anas platyrhynchos (mallard duck)	NOAEL	14.1	Old: DAR 2000 & Review report Chlorothalonil	Shults et. al. (1988) CA 8.1.1.3/06 (KIIA 8.1.3)
Colinus virginianus (bobwhite quail)	NOAEL	**10.1**	Old: DAR 2000 & Review report Chlorothalonil	Shults et. al. (1988) CA 8.1.1.3/08 (KIIA 8.1.3)
Formulation (Rover)				
Acute oral (gavage)				
Anas platyrhynchos (mallard duck)	14d LD_{50}	> 2000	New Study	Fairley, C. (1985); CP 10.1.1.1/01
Mammals				
Chlorothalonil				
Acute Oral				
Rat	LD_{50}♂ LD_{50}♀	> 5000	Old: DAR 2000 & Review report Chlorothalonil	Moore, 2000; CA 6.2.1/01
Rat	LD_{50}♂ LD_{50}♀	> 10000	Old: DAR 2000 & Review report Chlorothalonil	Shults, 1981a; CA 6.2.1/02
Rat	LD_{50}♂ LD_{50}♀	> 5000	Old: DAR 2000 & Review report Chlorothalonil	Cummins, 1988a; CA 6.2.1/03
Mouse	LD_{50}♂ LD_{50}♀	> 5000	Old: DAR 2000 & Review report Chlorothalonil	Cummins 1989; CA 6.2.1/04
Rat	LD_{50}♂ LD_{50}♀	> 5000	Old: DAR 2000 & Review report Chlorothalonil	Apte, 1992; CA 6.2.1/05
Mouse	LD_{50}♂ LD_{50}♀	> 5000	Old: DAR 2000 & Review report Chlorothalonil	Apte, 1992; CA 6.2.1/06

Species		Endpoint	Value [mg a.s./kg bw (/d)]	Old/new dossier	Reference
Reproduction					
Rat	$NOAEL_{parental}$ $NOAEL_{developmental}$ $NOAEL_{reproductive}$	< 22.6 22.6 145.1[a]		Old: DAR 2000 & Review report Chlorothalonil	Lucas et. al., 1990; CA 6.6.1.1/01
Rat	$NOAEL_{parental}$ $NOAEL_{developmental}$ $NOAEL_{reproductive}$	< 32.7 <32.7 261[a]		Old: DAR 2000 & Review report Chlorothalonil	Myers et. al., 1995; CA 6.6.1.1/02
Developmental					
Rabbit	$NOAEL_{parental}$ $NOAEL_{developmental}$	10 20		Old: DAR 2000 & Review report Chlorothalonil	Wilson, 1988i; CA 6.6.2.4/01
Rabbit	$NOAEL_{parental}$ $NOAEL_{developmental}$	10 10		Old: DAR 2000 & Review report Chlorothalonil	Meyers, 1994c; CA 6.6.2.4/02
Rat	$NOAEL_{parental}$ $NOAEL_{developmental}$	<25 100		Old: DAR 2000 & Review report Chlorothalonil	Mizens, 1983; CA 6.6.2.4/03
Rat	$NOAEL_{parental}$ $NOAEL_{developmental}$	80 <80		Old: DAR 2000 & Review report Chlorothalonil	Meyers, 1994b; CA 6.6.2.4/04
Mouse	$NOAEL_{parental}$ $NOAEL_{developmental}$	100 100		New Study	Farag et al., 2006 CA 8.1.2.2/01
metabolite R182281 (SDS-3701)					
Acute Oral					
Rat	LD_{50}	332		Old: DAR 2000 & Review report Chlorothalonil	Wazeter, 1971a CA 6.8.1-6.2.1
Rat	LD_{50}♂ LD_{50}♀	422 242		Old: DAR 2000 & Review report Chlorothalonil	Hastings, 1973 CA 6.8.1-6.2.3
Rat	LD_{50}	50-300		New study	Beerens-Heijnen, 2005 CA 6.8.1-6.2.4
Reproduction					
Rat	$NOAEL_{parental}$ $NOAEL_{developmental}$ $NOAEL_{reproductive}$	3 1.5 6		Old: DAR 2000 & Review report Chlorothalonil	Ford et. al., 1982c; CA 6.8.1-6.6.1.1
Rat	$NOAEL_{parental}$ $NOAEL_{developmental}$ $NOAEL_{reproductive}$	269 911 911		Old study: DAR (2000)	Lucas et. al., 1993; CA6.8.1-6.6.1.2
Formulation A14111B					
Acute oral (gavage)					
Rat	LD_{50}♀	3045		New study	

In Volume 1, section 2.9.1 an overview of the available endpoints for birds and mammals is given and an explanation of the selected endpoints. This discussion is reproduced below for ease-of use:

Discussion of endpoints to be used in the risk assessment

Birds

The endpoints for birds used in risk assessment are as shown in **bold** in the table above, and are the same as those in the original DAR for chlorothalonil. The applicant used values of 16 mg/kg bw/d and 5 mg/kg bw/d, based on the default conversion factor from the EFSA (2009) Guidance, however, the RMS uses the values from the original DAR, which were based upon actual food consumption in the studies.

After the commenting period the notifier argued that the reproductive endpoint for SDS-3701 from the study in mallard should be raised to the next higher dose of 14.1 mg/kg bw/d, as the only effect a the lower dose of 6.96 mg/kg bw/d was an effect of 8% on egg shell thickness. Since the study was reported, the EFSA bird and mammal guidance has been issued and gives more information on the interpretation of the studies, specifically on determining NOELs and NOAELs. Consideration should be given as to whether a significant effect giving a NOEL is biologically relevant and whether a NOAEL is more appropriate. Eggshell thinning is specifically cited in EFSA (2009) as such an example. This is also stated in the draft Guidance on Biological Relevance (EFSA March 6, 2017), which refers to information from the EFSA Guidance (2009) and Blus, L. 2003[2], "It is believed that the biological [sic] relevant percentage of egg shell thinning starts with 18%." A graph is presented which suggests that increased egg cracking only occurs at more than 18% egg shell thinning and that even higher than this is the relevant endpoint value (considering the % of cracked eggs which is relevant to have an population level effect. The graph is reproduced below, taken from Figure 5 of Annex K of the draft Guidance on Biological Relevance (2017).

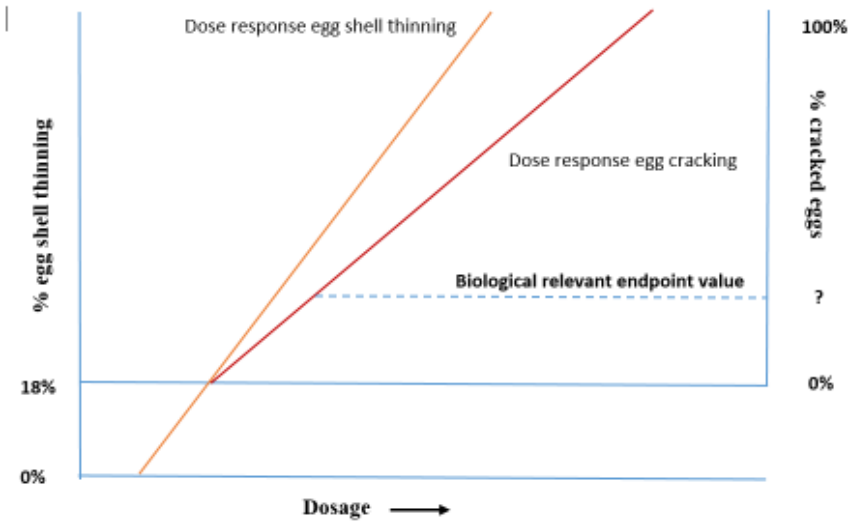


Figure 5: Relation between egg shell thickness (orange line) and cracked eggs (red line). The dashed line is the line for effecting the reproduction of a bird species (e.g. when is the number of cracked eggs too much for maintaining a stable population).

Taking these facts into consideration, the RMS considers the endpoint of 14.1 mg/kg bw/d to be the biologically relevant endpoint from this test.

[2] Blus, L., 2003. Handbook of ecotoxicology: Organochlorine pesticides. Chapter 13. 2nd ed. CRC Press LLC, Boca Raton.

In addition, after the commenting period a re-analysis of the study in bobwhite quail showed a lower endpoint of 10.1 mg/kg bw/d to be the most appropriate, based on decreased egg production.
This means that the endpoint from the study with quail, of **10.1 mg/kg bw/d**, is actually the lowest reproductive endpoint in the tested species and is therefore the appropriate biologically relevant reproductive endpoint to use for SDS-3701.

The notifier also argues that the reproductive endpoint for birds from the parent chlorothalonil should be raised. They suggest considering the studies of Shults (KIIA 8.1.3/03) and Redgrave (KIIA 8.1.3/05) together, and propose using the NOAEL from Shults (158 mg/kg bw/d) in the risk assessment. To support this argumentation they point out that the effects on egg production at the highest tested dose in Redgrave are not statistically significant, and occur at a dose lower than the NOEAL in the study by Shults et. al. Furthermore, they point out that the eggs per female in the study by Redgrave was 82% of control, but was still 51 eggs per female, and that in the Shults study the control only produced 43 eggs per hen, with 93% of control at the NOEAL concentration and 67% of control at the next highest dose.

Looking at the study by Shults et. al., there appears to be a clear trend toward decreased reproductive viability throughout the doses (Table 8.1.1.3-4 is reproduced from the CA document for ease of consideration):

Table 8.1.1.3-4: Summary of effects of technical chlorothalonil on fecundity of bobwhite quail during the exposure period

Reproductive parameter	Test concentration (ppm ai)			
	Exposure Period			
	0	1000	5000	10000
Eggs laid	690	644	403	48
Eggs cracked	21	33	18	0
Eggs set	543	511	315	28
Viable embryos	505	455	264	6
Live 3 week embryos	498	451	262	6
Hatchlings	473	423	225	4
14 day old survivors	452	405	175	1
Eggs laid/hen	43	40	29	4
Eggs laid/hen/day	0.63	0.58	0.42	0.05
14 day old survivors/hen	28	25	13	0
Eggs laid/max laid (%)	72	67	48**	6**
Eggs cracked /Eggs laid (%)	3	6	4	0
Viable embryos/set (%)	93	86	83	29**
Live 3 week old embryos/viable (%)	98	99	99	100
Hatchlings/3-week (%)	95	94	85	67**
14 day old survivors/hatch (%)	95	96	73**	25**
Hatchlings/set (%)	87	80	71*	19**
14 day old survivors/set (%)	83	77	54**	4**
Hatchlings/max set (%)	60	54	33**	1**
14 day old survivors/max set (%)	58	52	26**	0**

* Difference from the control statistically significant at $p < 0.05$

** Difference from the control statistically significant at $p<0.01$

Since the trend is clear, in fact it could be considered that there is no NOEL from this study, however, since the eggs/hen are 93% of control at the dose of 1000 ppm (158 mg/kg bw/d), it is questionable whether the slight effect at this level could be considered biologically relevant. It is also not statistically significant. Thus, the RMS concludes that the appropriate NOAEL of 158 mg/kg bw/d was chosen from this study.

The reproductive results from Redgrave are also reproduced below (from Table 8.1.1.3-10 in the CA).

Table 8.1.1.3-10: Summary of effects of technical chlorothalonil on fecundity of bobwhite during the exposure period

Reproductive parameter	Test concentration (ppm ai)			
	0	**40**	**160**	**640**
Eggs laid per female	62.0	62.4	68.9	51.0
Eggs damaged	55	23	58	36
Eggs damaged of eggs laid (%)	3.7	1.6	3.7	3.2
Mean egg shell thickness (mm)	0.20	0.21	0.21	0.20
Eggs set	1304	1288	1364	985
Viable embryos	1132	1226	1254	881
Viable embryos of eggs set (%)	87	95	92	89
Live 3-week embryos	1079	1209	1197	841
Live 3-week embryos of viable embryos (%)	95	99	95	95
Normal hatchlings	956	1070	1059	739
Normal hatchlings of live 3-week embryos (%)	89	89	88	88
Normal hatchlings of viable embryos (%)	84	87	8	84
14-day old survivors	888	987	950	684
14-day old survivors of normal hatchlings (%)	93	92	90	93
14-day old survivors of eggs laid (%)	60	69	61	60
14 day old survivors per female	37.2	42.8	42.2	30.4
Chick bodyweights at hatching (g)	6.8	7.0	6.7	6.8
Chick bodyweights at 14-days (g)	24	25	24	25

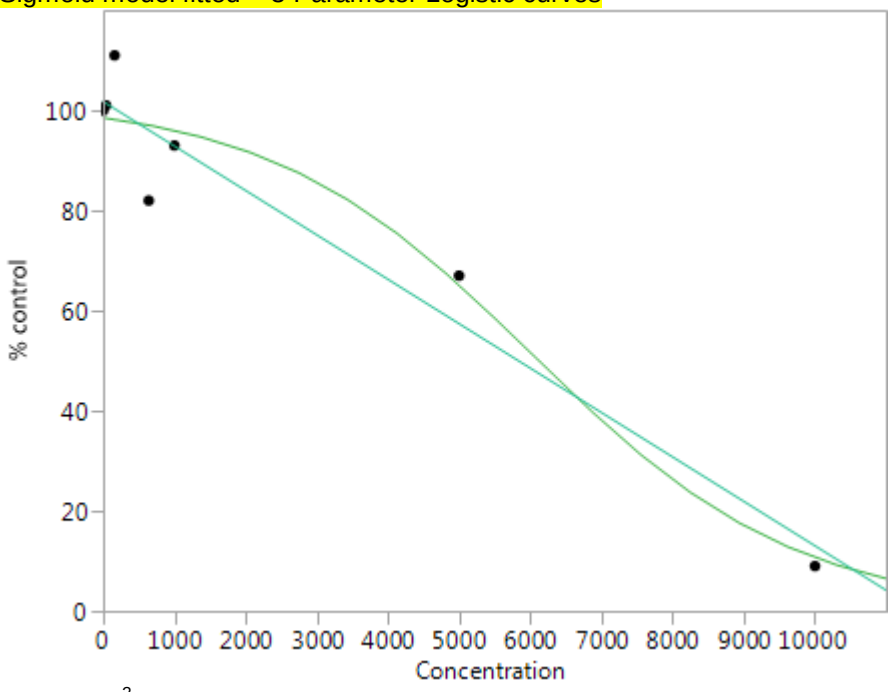
A clear trend is seen at 640 ppm in eggs/hen and the related variables, but other reproductive parameters including embryo viability and survival, hatchling survival, 14d survival, and eggshell thickness do not appear to be affected. The eggs laid per hen is approximately 82% of control. The effect on eggs per hen is not statistically significant, however, the study author considerd it treatment related, thus the NOEL was set to the next highest dose (equivalent to 14.1 mg a.s./kg bw/day).

Considering the similarity of design of the two studies, it seems acceptable to combine them. However, in order to interpret the egg production data (which appears to be the most sensitive) the control egg production in the two tests must be normalized. The notifier proposed doing this by setting the control values to 100 and using the percentage of control as the value for the other doses. This resulting in the following values for the most sensitive endpoint, eggs/hen, which the notifier calculated as such:

ppm	% control
0	100
40	115
160	113
640	82
1000	89
5000	46
10000	0

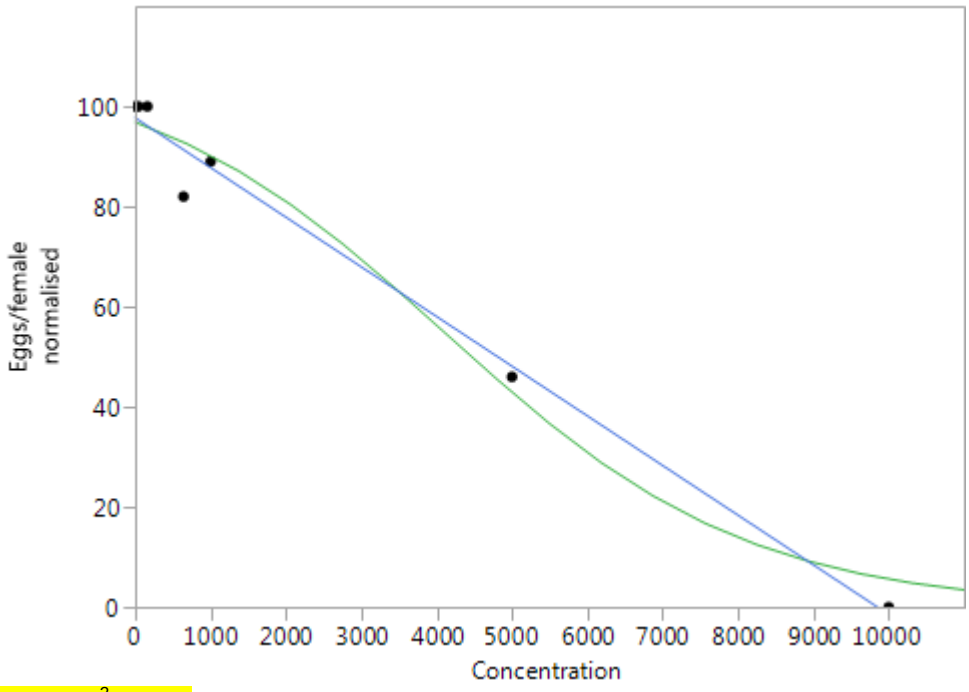
From this, the notifer used JMP® Statistical Software 12.0.1 ©SAS Institute Inc to fit both linear and sigmoid curves to the adjusted combined data, resulting in the following:

Sigmoid model fitted = 3 Parameter Logistic curves



Sigmoid R^2 = 94.2

The notifier also graphed the data setting the two values that were over 100% (i.e. where egg production per hen was greater than control) to 100, which resulted in a better curve fit.



Linear R^2 = 98.6
Sigmoid R^2 = 97.6

Therefore, the notifier concludes that the curves suggest that the value at 1000ppm (89% of control) is the most appropriate NOEC as the lines pass right through/close to it, whereas the value at 640 ppm (82% of control) does not fit in the overall curves.

The RMS considers it acceptable to merge the two studies, and to adjust the data in the manner proposed by the notifier in order to compare them. Looking at the adjusted data, it could be considered whether the reduction in eggs/hen at the two middle doses is biologically significant, and if so, which dose would be the most conservative value for use in the risk assessment.

The RMS has used the data for eggs/hen for both studies and calculated the following values, including normalizing those values above 100% to 100:

ppm	mg/kg bw/d	% control
0	0	100
40	3.6	100
160	14.1	100
640	58.2	82.3
1000	158	93
5000	767	67.4
10000	1540	9.3

The RMS has tested this data using BMDS. The adjusted data was used considering relative deviation, and using the mg/kg bw/day from each study, as the translation of ppm to mg/kg bw/day was not the same for the two studies, and thus this was deemed the most accurate. This calculation resulted in $BMDL_5$ of 119 mg/kg bw/day, fitted in the polynomial model 4, relative deviation. A report and graph of the model can be found in Appendix I. This model was chosen because it showed the lowest BMDL5, which was the recommendation of the BMDS Wizard, according to the US EPA technical guidance on benchmark dose modelling (2012). It was also the model for which the lower doses were modelled most accurately, according to the visual inspection of the RMS.

Conservatively, therefore, a 5% effect on number of eggs per hen could be found at a dose of 119 mg/kg bw/day. A conservative NOEAL therefore would be the highest dose in the Redgrave study, 58.2 mg/kg bw/day. The RMS also calculatd a $BMDL_{10}$ (the same model was most appropriate), which was 238.2 mg/kg bw/day.

Generally speaking, the test design and dose spread are not ideal for modelling, however, the benchmark dose modelling software did present a number of viable models, and the RMS was able to chose the most relevant. The RMS therefore considers the dose of 58.2 mg/kg bw/day a conservative NOAEL. It could be discussed by the experts whether a dose of 158 mg/kg bw/day might be the most biologically appropriate value to use, as the modelling indicates it is a dose which results in a slightly over 5% effect on eggs per hen, but significantly lower than 10% effect. The RMS will present the risk assessment considering these two values, as well as the $BMDL_5$ (119 mg/kg bw/d).

Mammals

In the original DAR (2000), the wild mammalian endpoint for chronic/reproductive toxicity was based on the value of 10 mg/kg bw/d from Meyers, 1994c; CA 6.6.2.4/02, a developmental toxicity study in rabbits. The notifier argues that:

…the LOAEL was defined as 20 mg/kg bwt/day for dams based on a significant reduction in food consumption and bodyweight gain and as 20 mg/kg bwt/day for developmental effects based on an increased incidence of rudimentary ribs, reduced sternebrae and other indications of delayed ossification of the skeleton. A slightly higher incidence of post-implantation loss at 20 mg/kg/day in the Myers study was considered to be within the incidence normally seen in rabbits.
A choice of 10 mg/kg bwt/day as the NOEC for ecological risk assessment is considered inappropriate. The developmental finding of reduce sternebrae and rudimentary ribs seen in the Myers study are considered likely attributable to delays in the normal ossification pattern of the skeleton. An effect on ossification was seen at the same dose level in the Wilson study. Such delays are often seen in highly labile areas of the skeleton in association with maternal toxicity. They are considered transitory[3] and do not impact survival of the young. Hence such finding would have no consequence on population dynamics and does not provide a basis for an ecologically relevant endpoint.
The choice of a developmental study to derive an ecologically relevant endpoint for long term risk assessment is further considered inappropriate because the study uses gavage dosing. Animals receive a bolus dose directly into the stomach once per day. This method of exposure can result in different levels of toxicity to those seen after dietary dosing which is clearly more representative of repeated wild mammal exposure.

Several developmental toxicity studies are available in the mammalian toxicology section (see Volume 1, Table 2.6.6-1). While we do not agree with the notifier that it is not possible to set an endpoint for use in wild mammal risk assessment based upon a study performed via gavage dosing, we agree that this can result in more conservative endpoints. Nonetheless, the developmental toxicity studies look at different endpoints than are investigated in the multi-generation studies, and may therefore provide better information on potentially relevant effects of the tested substance. The conservativeness of the endpoint and the relevance of the effect should be weighed.

In this case, none of the unique effects of the developmental toxicology studies were seen at a dose of 10 mg/kg bw/d, however, effects were seen at 20 mg/kg bw/d (extra ribs, abnormal sternae). The RMS agrees with the notifier that these effects are not ecologically significant, as they are not likely to effect the survival or robustness of the offspring. Furthermore, the acute effects on feeding in dams seen in Myers (1994c), are considered transitory and did not have a significant effect on bodyweight. Since there were no effects on development at the highest tested dose in the developmental toxicology studies (effects on dams were seen at 10 mg/kg bw/d, relating to anorexia), and since none of the other long-term oral tests show effects on bodyweight (at doses less than 100 mg/kg bw/day) or other

[3] Palmer AK (1968) Spontanous malformations in the New Zealand White rabbit: The background to safety evaluation tests Lab. Anim. 2, 195-206

relevant toxicities, we agree that the endpoint from the multigeneration study is most relevant for consideration for the ecotoxicological risk assessment.

Regarding the endpoint from the multigeneration study, the notifier states the following:

A relevant endpoint can be derived from the two generation study in the rat (Lucas and Killeen 1990). This study used dietary dose levels of 0, 500, 1500 and 3000 mg/kg diet (equivalent to 0, 22.6, 68 and 145 mg/kg bwt/day). There was no effect of chlorothalonil on fertility, litter size, pup survival or development. A consistently lower bodyweight was noted in pups at 3000 ppm in each of 2 litters in both generations at 21 days post partum. Although the maximum reduction compared to concurrent control values was 14% in the F1b litter and despite the fact that these animals went on to produce normal litters of their own, the consistency of this effect leads to the conclusion that this is potentially an ecologically significant effect. At 1500 mg/kg diet a statistically significant reduction of 8% compared to concurrent control weight was seen only in the F1b litter. The F1a litter and both F2 litters showed no significant difference from control values. There is, therefore, no consistent effect on litter weight up to day 21 post partum at 1500 mg/kg diet and this is considered to be the ecologically relevant NOEC.

The appropriate NOEC for wild mammal risk assessment of chlorothalonil is therefore 1500 mg/kg diet (68 mg/kg/bw/day).

The robustness of this endpoint is reinforced with a literature study on maternal and developmental toxicity in mice reviewed in MCA Section 5 supplement (Farag 2006), where maternal toxicity was observed at 400 and 600 mg/kg bw/day including weakness and depressed maternal activity, and reduced body weight and body weight gain. At 400 and 600 mg/kg bw/day, the number of live foetuses, early resorptions and mean foetal weight was significantly reduced. The NOAEL for maternal and developmental toxicity in this study was 100 mg/kg bw/day.

The RMS not agree with the proposal of the notifier, as although the F1a pup weight decrease had not reached siginificance, there was a clear trend, and the F1b pup weight was already statistically significantly decreased. Further, in a 2nd multigeneration study, the developmental NOAEL was set at < 32.7 mg/kg bw/d, based on gastric changes in pups in all generations at the lowest tested dose (of 32.7 mg/kg bw/d). Although these gastric changes are not considered relevant for ectoxicological risk assessment, they do show effects which may, after prolonged exposure, result in weight effects occurring at doses greater than 22.6 mg/kg bw/d. Pup weight effects were seen in the 2nd study at the next highest dose of 100 and at the highest tested dose of 261 mg/kg bw/d. Taken together, the RMS concludes that the endpoint from the 1st multigeneration study in rats, of **22.6 mg/kg bw/d** should be used in risk assessment.

The risk assessment is based on the following endpoints:

Chlorothalonil

Birds acute: LD50 > **2000 mg a.s./kg bw**

Birds reproduction: NOEL **58.2, 119 or 158 mg a.s./kg bw/d**

Mammals acute: LD_{50} **> 5000 mg a.s./kg bw**

Mammals reproduction: NOAEL **22.6 mg/kg bw/d**

SDS-3701

Birds acute: LD50 **158 mg a.s./kg bw**

Birds reproduction: NOEL **10.1 mg a.s./kg bw/d**

Mammals acute: LD_{50} **242 mg a.s./kg bw**

Mammals reproduction: NOAEL **1.5 mg/kg bw/d**

B.9.2.1.1 Metabolites of chlorothalonil

The metabolite SDS-3701 (4-hydroxy-2,5,6-trichloroisophthalonitrile) is a relevant soil and plant metabolite which is formed above 10% of parent. The RMS has also investigated the other chlorothalonil metabolites: all tested metabolites are less toxic than the parent in mammalian toxicology studies. Further, in the plant residue studies, only SDS-3701 was present in significant amounts. In a hen feeding study with chlorothalonil, SDS-3701 was the major identified metabolite. Taken together, metabolite SDS-3701 is the only metabolite that should be considered in the birds and mammals risk assessment. Due to its lower toxicological endpoints SDS-3701 is considered in the risk assessment.

As a plant metabolite, it is necessary to derive a concentration of SDS-3701 for use in the risk assessment. In the original DAR the following was concluded:

The conclusions from Chapter B.7 Residue data is that SDS-3701 is major metabolite on plant foliage: max. 14% of the residue present at 14 days post-harvest interval (PHI). In general, the amount of SDS-3701 is 2-20 times lower than the amount of chlorothalonil, but in one event the amount of SDS-3701 (12%) was higher (4% chlorothalonil).

The notifier states that:

At the point where the maximum 14% of the residue was SDS-3701, the actual residues of chlorothalonil and SDS-3701 were 5.8 and 1.1 mg/kg respectively. Thus the maximum measured residue of SDS-3701 in this study from 3 weekly applications of 2.33 kg a.s./ha was 1.1 mg/kg. In calculating the exposure using the application rate, maximum formation percentage, using the mean RUD for cereals (worst case) and correcting for mass (0.93) the exposure is over estimated as shown in the table below:

Table 9.2-1: Overestimating theoretical exposure to SDS-3701 based on maximum %age of residue and chlorothalonil RUD

Test substance	*Mean RUD (grass and cereals)*	*Maximum %age of residue as SDS-3701 (%)*	*Application rate of chlorothalonil (kg a.s./ha)*	*Mass correction*	*Exposure (mg/kg)*
SDS-3701	*54.2*	*14*	*2.33*	*0.93*	*16.4*

Measured residue values are available from the plant metabolism study above from which the maximum formation rate was taken (1.1 mg/kg from 3 x 2.33 kg a.s./ha applications). In addition, a huge USA residue database was summarised in Edwards 2001 (Report No. ERA3273, R44686/3287), see the Table below from this report.

Table 9.2-2: 50th and 90th percentile estimates of chlorothalonil and SDS-3701 using the crop residue database. (from Edwards 2001)

Vegetation type	*Total rate (kg/ha)*	*Measured chlorothalonil residues (mg/kg fresh weight)*		*Measured SDS-3701 residues (mg/kg fresh weight)*	
		50th percentile	*90th percentile*	*50th percentile*	*90th percentile*
Short grass	*5.5-49*	*250*	*1700*	*1.8*	*5.2*
Long grass	*10.5*	*4.4*	*23.3*	*0.05*	*0.17*
Leafy crops	*9-15.2*	*0.23*	*5.9*	*0.005*	*0.03*
Vegetable flowers	*4.7-12.9*	*1.8*	*5.8*	*0.013*	*0.09*
Fruit	*2.0-53*	*0.43*	*5.1*	*0.015*	*0.025*
Seed (single sample of grass seed)	*1.5-10.5 (4.5)*	*0.015*	*0.015 (43.5)*	*0.015*	*0.015 (0.49)*
Dry vegetation = insect	*1.5-9*	*0.47*	*13*	*0.18*	*0.86*

Thus it is clear that exposure to residues of SDS-3701 will be low and it is proposed that a default worst-case residue value of 1mg/kg is used in the initial risk assessment. The value of 1 mg/kg is considered conservative based on the application used in the plant metabolism studies of 3 x 2.33 kg a.s./ha at least 7 days apart, compared to this submission for 2 x 0.75 kg a.s./ha at least 14 days apartand 1 x 1 kg a.s./ha. The total amount applied under this evaluation is approximately 20% that applied in the plant metabolism study.

~~A summary of the report Edwards, 2001, was submitted by the notifier and is presented in CA Section B.9.1.6. The report itself was submitted for an addendum to the original DAR (Addendum XX)[4] and re-submitted with this dossier. The residues trials analyzed for the report were performed in the United States, and the exact conditions for each trial are unknown (i.e. weather conditions, parts sampled, etc.). However, a large number of trials were analyzed to determine residues of chlorothalonil and SDS-3701 in several crops (see Table 1 of the summary, located in CA 9.1.6). The residues section shows that levels of SDS-3701 were <5% of TRR in several studies where measurements were taken on d0 and decreased in later measurements. Further, it is noted that SDS-3701 was found at up to 35% of TRR in the hen feeding study with chlorothalonil (see Section B.7.2.2.3), suggesting that it is~~

[4] These data were used to refine the risk to birds and mammals using the measured residues levels to estimate dietary exposure.

formed at a relatively high level in bird metabolism and it is the first metabolite in the mammalian metabolism studies. SDS-3701 was the major plasma metabolite found in the rat metabolism studies, accounting for 28-37% of radioactive AUC. Considering the fact that chlorothalonil is poorly absorbed (only about 20% absorption), it is probable that the metabolite SDS-3701 contributes significantly to the toxicity of the parent, chlorothalonil, in both birds and mammals. Nonetheless, considering the comparative toxicity of SDS-3701, the RMS will perform a separate risk assessment for SDS-3701. Considering the large amount of residues data analysed in Edwards (2001), and the relatively low levels of SDS-3701 found in the residues section (CA B.7), the RMS will use the 90th and 50th %-ile measured residues of SDS-3701 in short grasses, which was the crop with the highest measured residues levels for SDS-3701, i.e. short grass (from Table 2 of the study summary, reproduced below).

Table 2 (from Edwards, 2001): 50th and 90th percentile estimates of chlorothalonil and SDS-3701 using the crop residue database.

Vegetation type	Total application rate (lb/ha = 0.4536 kg/ha)*	Measured chlorothalonil residues (mg/kg fresh weight)		Measured SDS-3701 residues (mg/kg fresh weight)	
		50th percentile	90th percentile	50th percentile	90th percentile
Short grass	5.5-49 (= 2.5-22)	250	1700	1.8	5.2
Long grass	10.5 (= 4.8)	4.4	23.3	0.05	0.17
Leafy crops	9-15.2 (= 4.1-6.9)	0.23	5.9	0.005	0.03
Vegetable flowers	4.7-12.9 (= 2.1-5.9)	1.8	5.8	0.013	0.09
Fruit	2.0-53 (= 0.9-24)	0.43	5.1	0.015	0.025
Seed (single sample of grass seed)	1.5-10.5 (4.5) (= 0.7-4.8)	0.015	0.015 (43.5)	0.015	0.015 (0.49)
Dry vegetation = insect	1.5-9 (= 0.7-4.1)	0.47	13	0.18	0.86

*The table and the body text of the document referred to by the notifier mention application rates in kg a.i./ha, but according to the appendices the application rates mentioned in the table are in lb a.s./ha. It is not known which is correct. To ensure a worst case approach, Ctgb recalculated the values to kg a.s./ha.

This level is considered conservative and is therefore expected to cover the several uncertainties involved in the residues study, as well as in the derivation of an appropriate exposure level to the metabolite. Further, it is not expected that the residues will be higher than this in other food items, as residues in fruits, flowers and seeds were much lower than this. Further, the information provided by the notifier in Edwards, 2001, confirm that the residues levels of SDS-3701 are relatively consistant,

independent of dose rate and that they do not increase over time. The notifier argues that the residues values can be used independent of dose rate, considering the lack of correlation of the residues levels with dose rate of cholorothalonil in the analysis of Edwards, 2001. The RMS notes that the pattern of residues of SDS-3701 seems to closely follow the pattern of chlorothalonil residues, regardless of application rate. For fruit, where the largest number of residues measurements are available, the residues levels seem to slightly follow application rate for the final application (see figures 15 and 16, of the original report). However, this pattern was not seen in the other groups, including the short grasses, where the highest residues were seen at a final application rate of ~10.5 lb/A, and similar or slightly lower levels were seen at ~16.5 lb/A (see figures 9 and 10 of the original report). Taken together, the RMS agrees to use the 50th and 90th %-ile residues levels to estimate the total exposure of birds and mammals to the plant metabolite SDS-3701, however, we welcome member state opinions on this issue.

An uncertainty table discussing the choice of exposure level for the metabolite, SDS-3701, is presented below.

Table 9.2.1: Uncertainties and weight of evidence for derivation of exposure levels for SDS-3701 in bird and mammal food items

Consideration	Source of uncertainty	Effect on conservativeness	Conclusion
Residues trials analyzed in Edwards, 2001	Climatic conditions unknown	-	The climatic conditions for each of the trials is unknown, therefore increasing the uncertainty in assuming these to be representative of the EU.
Residues trials analyzed in Edwards, 2001	Details of each study unknown (plant parts, etc.)	-	Some details of the residues trials themselves cannot be checked, which means that potentially some trials would be less acceptable according to current standards or show large differences from the use in question. This increases the uncertainty in volved in the use of the estimated residues from the trials.
Residues trials analyzed in Edwards, 2001	High number of trials	+/-	A greater number of trials increases the chance that a more realistic residue level can be determined (i.e. variability is more visible and taken into account in residues

			~~estimations).~~
~~Residues trials analyzed in Edwards, 2001~~	~~Use of estimated residues from highest measured crop for all food items~~	~~+/-~~	~~The use of the residues estimated from short grasses to cover all bird and mammal food items increases the conservativeness of the risk assessment by over-estimating the residues levels in other food items. For herbivorous mammals this aspect is less applicable. Further, there are no data to indicate residues levels on arthropods, though in the RAR addendum the notifier stated that these could be estimated using the measurements on nut shells. The RMS does not agree that this adequately represents the residues on arthropods, but considering the large amount of data in quite different matricies considers the conservative values used in the RA to most likely cover the residues on arthropods.~~
~~Residues trials analyzed in Edwards, 2001~~	~~Use of residues trials performed not according to the current GAP (i.e. higher application rates and shorter intervals)~~	~~+~~	~~According to the RMS residues expert, it is common practice to assume that residues levels will be lower for applications at lower rates and with longer intervals.~~
~~Residues trials analyzed in Edwards, 2001~~	~~Use of measured residues without normalization to application rate 1 kg/ha.~~	~~+/-~~	~~The residues levels are independent of total and final application rate. No residues higher than 7 ppm are measured in any plant group at any application rate.~~
~~Presence of SDS-3701 in both hen feeding and mammalian metabolism studies~~	~~SDS-3701 probably contributes a large portion of the measured toxicity of the parent molecule~~	~~+~~	~~Performing a separate risk assessment for SDS-3701is considered relatively conservative since SDS-3701 is a major metabolite of chlorothalonil in both birds and mammals~~

			~~and chlorothalonil itself is not well absorbed/available.~~
~~Conclusion~~	~~The use of the 50th and 90th%ile residues level of SDS-3701 from the analysis of many residues studies presented by the notifier in Edwards, 2001, is considered acceptably conservative to cover the potential risk to birds and mammals from the metabolite SDS-3701.~~		

In response to numerous comments on the subject of the level of SDS/3701 to be used in the risk assessment, the notifier submitted several reports and an updated statement on the residues levels to be used in risk assessment. They agreed that the residues trials in Edwards are uncertain, but refer to the residues trials performed for this dossier to address the potential residues in plant matter.

"… there are trials specific to the EU GAPs in the MCA Section 6, where residues of SDS-3701 has been analysed. In the cereal trials, as well as the grain being sampled as the commodity for human consumption, the vegetation has been sampled and analysed, as whole plant or straw, as it used for livestock feed. As such, it provides a database suitable for estimating residues in food items for birds and mammals.

There were a total of 64 trials from 2011- 2014, 32 in each of wheat and barley, sampled from application at BBCH30 through to harvest. Residues of SDS-3701 tended to be highest at Day 0, after application, but not always. Residues ranged from <LOD (0.01/0.02 mg/kg) to a maximum of 1.1 mg/kg in one barley trial, which was actually at a PHI of 53 days. The second highest residue was on day 0 in a wheat trial at 0.74 mg/kg. There were a total of 241 measurements of SDS-3701, mean 0.12 mg/kg, 90th %ile 0.38 and maximum of 1.1 mg/kg.

Thus the proposal is, rather than use a mean or 90Th%ile residue, to use the maximum measured residues of 1.1 mg/kg SDS-3701 in the initial risk assessment for values in vegetation for both cereals and tomatoes. Non-target vegetation in both crops will likely be significantly lower due to interception, but this will only be considered if refinement is necessary.

… in tomatoes, the fruit could be considered a food item for frugivorous birds. In the 16 tomato residue trials conducted in 2014 and 2015 across Europe, applied at 1 kg chlorothalonil/ha, a residue of R182281 was measured only once at 0.02 mg/kg, all other values were <LOQ (0.01 mg/kg). This is confirmed by the tomato plant metabolism study, where following 3 applications at 2.33 mg/kg, the highest residue in fruit was measured at 0.04 mg/kg, one day after the last application, declining to 0.02 mg/kg at 14 days. From the US residue trails summarised in Edwards 2001, the 50% ile residue in fruit was 0.015 mg/kg, and from higher application rates than in this submission. Thus the highest measured residue from the EU residue trails of 0.02 mg/kg is worst-case for exposure levels in fruit."

The RMS agrees with the conclusions of the notifier regarding the residues shown in the trails in CA B6. Further discussion from the residues section is presented below.

The notifier also referred to the Edwards report once again, in order to address other food items for birds and mammals. To support this they submitted two of the residues studies from that report, which were evaluated by the RMS and are presented in Section XXX.

"One further consideration is the representativeness of the crop to represent the vegetation the generic focal species is supposedly eating. This will be vegetation beneath the crop canopy, described in the EFSA bird and mammal guidance appendices as non-grass herbs. The majority of residue data focuses on crops, however the US data summarised in Edwards (2001) includes a short grass category which is the closest to the sort of vegetation typical of that likely to be below the crop canopy, indeed when it comes to the mammal risk assessment, grass is the diet assumed at tier 1 for the vole and so it is clearly representative. The data within the short-grass category comes from 2 reports, summarised below to give an indication of the level of residues which might be expected on short grass as an indication that the level used in the assessment is conservative.

The first report has the residues translated into an equivalent RUD, normalised to a 1 kg/ha application. The second report shows similar levels of SDS-3701, ranging from non-detects (< 0.01 ppm) to a maximum mean measured of 6.19 ppm. However RUDs have not been calculated here, because it is not possible to substantiate the application rate. Although it is given as 3 applications at 16.335 lb ai/ac in a summary, which would be in line with US application rates on turf, it is not in the report and cannot be substantiated. Nevertheless it give WoE to the likely residues of SDS-3701 being low relative to the parent. The maximum residues of SDS-3701 are at ~ 6 ppm are approximately 400x below those of the parent at ~2500 ppm, which shows that a formation fraction of 14%, promoted as one way of calculating SDS-3701 residues is not appropriate. Furthermore this second report gives a measure of the decline of residues of the parent on turf, with 46% decline in one day, equivalent to a DT_{50} of approximately 1.1 days.

Residues of SDS-3701 in earthworms and soil dwelling arthropods would therefore be lower than the surrounding soil.

Certainly there is no evidence to suggest residues will be higher in insects than any other food items. Furthermore it should be stated that a risk assessment has been conducted for the maximum residues of the parent on insects and based on the bird and mammal guidance this should cover SDS-3701 as well. Thus, again in the first instance the same worst-case measured residue in plant food items of 1.1 mg/kg will be used for seeds, soil dwelling insects and worms, as required."

The RMS has evaluated the studies by King et. al. in short grasses and do not find them to be acceptable for use in risk assessment. However, as stated by the notifier, they do suggest that the levels of residues of SDS-3701 in short grasses are significantly lower tha the residues of parent, and support the notifier and RMS conclusion that the use of the TRR percentage as a formation percentage is too worst case.

In addition to the information presented by the notier, the RMS was asked to consult with the residues expert on various aspects of the risk assessment for the metabolite R182281 (SDS-3701), including the level to be used in the risk assessment, the decline of the residue of the metabolite, and the issue of combined exposure to the parent chlorothalonil and the metabolite. The RMS residues expert stated that:

(1) In the plant metabolism studies, which included measurements various parts of cereals, the residues level of 1.1 mg/kg fresh weight is by far the highest measured residue. The levels measured in plant material can be found in Tables CA B.7.3.3-4 and CA B.7.3.2-4, and show that most of the measurements were at least 10 times lower than that. The residues of SDS-3701 found in tomatoes are shown in Table CA B.7.3.1-4, and show that the maximum measured level of 0.02 mg/kg is an acceptable worst case value for tomatoes.

(2) The metabolite is very quickly turned into the next metabolite, probably virtually instantaneously, but in any case in less than one day. The residue dynamics of this metabolite are, however, not completely linear, as the levels are occasionally higher at later measurements than would be expected.

(3) The RMS residues considers it possible that there will be combined exposure to the parent and metabolite, however, as the parent decreases, the metabolite increases. The RMS residues has determined various ratios of parent to metabolite in the various commodities important for the risk assessment for human consumption. These may be found on page 148, above Table CA B.7.4-1. These are intended for consideration by the residues experts and may not be fit for purpose for the birds and mammals risk assessment, however, they do indicate that the levels of the metabolite are always lower compared to the levels of the parent. The RMS residues has calculated a total residues burden for various types of feed for livestock, the highest of which being 10.52 mg/kg total residues (9.9 mg/kg chlorthalonil plus 0.58 mg/kg SDS-3701 times 1.07 to account for molecular weight) in wheat straw. This is shown in Table CA B. 7.4-5.

Based on all of the above information, the RMS (ecotoxicology) has concluded the following. These conclusions should be discussed by the experts in the PRaPer.

(1) The residues of the metabolite in plant matter are unlikely to be greater than 1.1. mg/kg plant matter. This level can be used conservatively for all plant matter food items for birds and mammals.

(2) The residues level of 0.02 mg/kg suggests a very low level of the metabolite SDS-3701 in tomatoes. The RMS proposes using this as an exposure level in tomatoes for frugivorous birds and mammals.

(3) The residues of SDS-3701 in arthropods and seeds as food items is more difficult to estimate. Strictly speaking, there is no evidence to indicate that the metabolite forms in/on either arthropods or seeds. If it is assumed that the microbial population present in the soil is similar to that on seeds, it is possible that the metabolite forms on seeds. The $PEC_{soil,\ accumulative}$ for chlorothalonil and the metabolite SDS-3701 are similar, so a conservative estimation would give the level of SDS-3701 on seeds as equal to 0.351 mg/kg seed (equal to the $PEC_{soil,\ accum}$ of 0.351 mg/kg soil dw). This same estimation might be used for soil arthropods. The PEC_{accum} is conservatively calculated, assuming peak concentrations persist and are present in all locations. Since the metabolite does not appear to accumulate in organisms, using a PEC_{accum} (rather than the much lower PEC_{ini}) is also worst-case. For foliar arthropods, it is not clear

whether the metabolite is formed, as there is no evidence to indicate that it forms (or does not form) in/on arthropods. Taken together, the RMS considers the value of 1.1 mg/kg food to be a conservative estimation of the level of the metabolite that might be expected in seeds and arthropods. The RMS suggests using the value of 0.351 mg/kg food item for seeds and arthropods as a refined value.

(4) According to the residues expert and the information from the residues studies, the default residues decline DT_{50} value of 10 days is conservative for estimating the decline of the metabolite in plant food items for birds and mammals. The DT_{50} of the metabolite is unknown in arthropods. There is evidence to indicate that the DT_{50} of chlorothalonil in arthropods is relatively short (<3 days, from Schmidt, 2009), though this evidence is incomplete. If it was assumed that the ratio of the DT_{50}s in soil (chloro = 2.9 days, R182281 = 143.9 days, from Table CP 8.1-01), was the same for arthropods, it could be assumed that this DT_{50} is not conservative in arthropods. As a conservative measure, it could be assumed that there will be no decline of the residues of the metabolite in insect food items (i.e. f_{twa} = 1).

(5) There is no need to perform a combined risk assessment for the parent chlorothalonil and the metabolite SDS-3701, as the metabolite was present at relatively high levels in the rat and hen metabolism studies, indicating that the toxicity seen in the long term studies with the parent was already as a result of combined exposure to the parent and the plant metabolite SDS-3701, and that SDS-3701 is metabolized further by birds and mammals and thus does not accumulate. Furthermore, it would be difficult to estimate the exposure levels for a combined risk assessment. However, a risk assessment could be performed by combining the acute toxicity values according to the ratio used in the calculation of the RMS residues section to achieve the highest total body burden of both chlorothalonil and the metabolite (i.e. 17 to 1 chlorothalonil to SDS-3701) using the methodology recommended by the EFSA Guidance (2009). As a conservative measure, the total exposure rate of chlorothalonil could be used, however, it could be discussed whether it is more appropriate to use the maximum total residues burden calculated for ruminants by the residues expert (10.52 mg/kg). It should be noted that the maximum total residues in ruminants includes the highest measurement of SDS-3701, as well as many of the other non-zero measurements of SDS-3701, in straw.

Table 9.2.1-4 Acute LD_{50} for the mixture of the active substance and its metabolite

Test substance	Concentration of active substance in formulation A14111B (g/L)	Fraction of active substance and metabolite in the mixture[a]	Acute toxicity endpoint (mg/kg bw)	Fraction of active substance/LD_{50} for the active substance	LD_{50} mix (mg/kg bw)
Birds					
SDS-3701	unknown	0.059	158	0.00037342	**> 1184.95**
Chlorothalonil	400	0.941	> 2000	<0.0004705	
Total	unknown	1	-	<0.00084392	
Mammals					

Azoxystrobin	80	0.167	>5000	<0.000033	**>2367.83**
Chlorothalonil	400	0.833	>5000	<0.000167	
Total	480	1	-	<0.000200	

[a] using the estimation from the residues section of a 17 to 1 ratio of chlorothalonil to metabolite SDS-3701.

B.9.2.2 Dietary risk assessment for birds and mammals

B.9.2.2.1 Acute dietary risk to birds

Note: The risk assessment presented below has been adjusted according to the proposals above regarding endpoints and metabolite exposure. These will be discussed in an expert meeting, but reflect the RMS opinions.

Screening step

The acute 'daily dietary dose' (DDD) is calculated by multiplying the Shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha.

$$DDD_{multiple\ applications} = \text{application rate (kg a.s./ha)} \times SV$$

Table 9.2.2.1-1: Screening step – Acute risk to birds from A14111B

Compound	Crop group	Indicator species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg/ha)	MAF	DDD (mg a.s./kg bw/ day)	LD_{50}	TER
Chlorothalonil	Cereals	Small omnivorous bird	158.8	0.75	1.2	143	> 2000	14
	Fruiting vegetables			1.0	-	159		12.6
Chlorothalonil/ azoxystrobin	Cereals			0.90	1.2	172	> 2000	11.7
	Fruiting vegetables			1.2	-	191		10.5

Table 9.2.2.1-2: Screening step – acute risk to birds from exposure to SDS-3701

Compound	Crop group	Indicator species	FIR/bw	Residues level	MAF	DDD (mg a.s./kg bw/ day)	LD_{50}	TER
SDS-3701	Cereals	Small omnivorous bird	2.26	1.1	-	2.486	158	63.6
	Fruiting vegetables							

Table 9.2.2.1-3: Screening step – acute risk to birds from exposure to chlorothalonil + SDS-3701

Compound	Crop group	Indicator species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg/ha)	MAF	DDD (mg a.s./kg bw/ day)	LD_{50}	TER
Chlorothalonil	Cereals	Small omnivorous bird	158.8	0.75	1.2	143	> 1185	**8.29**
	Fruiting vegetables			1.0	-	159		**7.46**

As shown in the tables above, all uses pass in the acute screening step for dietary exposure. However, the potential exposure to the combination of the parent chlorothalonil and the metabolite R182281 (SDS-3701) does not pass in the screening step. A Tier 1 assessment is therefore necessary.

B.9.2.2.2 Long term dietary risk to birds

Screening step

The long term 'daily dietary dose' (DDD) is calculated by multiplying the Shortcut value (SV) based on the mean percentile residues by the application rate in kg a.s./ha and a default f_{TWA} of 0.53 taking into account a default residue decline (DT_{50}) of 10 days. In the event of multiple applications, a multiple application factor (MAF) is also included.

$$DDD_{multiple\ applications} = \text{application rate (kg a.s./ha)} \times SV \times 0.53 \times (MAF)$$

Table 9.2.2.2-1: Screening step – Long term risk to birds from chlorothalonil

Compound	Crop group	Indicator species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg/ha)	f_{TWA}	MAF	DDD (mg a.s./kg bw/ day)	NOEL	TER
Chlorothalonil	Cereals	Small omnivorous bird	64.8	0.75	0.53	1.4	36.1	14	**0.39**
	Fruiting vegetables			1.0		-	34.3		**0.41**

Table 9.2.2.2-2: Screening step – Long term risk to birds from exposure to SDS-3701

Compound	Crop group	Indicator species	FIR/bw	Maximum measured residues (mg/kg fresh weight)	f_{TWA}	MAF	DDD (mg a.s./kg bw/ day)	NOEL	TER
SDS-3701	Cereals	Small omnivorous bird	2.26	1.1	0.53	-	2.15604	10.1	7.67
	Fruiting vegetables								

As shown in the tables above, both chlorothalonil and the metabolite SDS-3701 should be further assessed at Tier 1.

Tier 1 assessment

Acute

Since the potential risk from the combination of the metabolite and active substance did not pass in the screening step, a Tier 1 assessmetn has been performed.

Table 9.2.2.2-3 Tier 1 – Acute risk to birds from exposure to chlorothalonil and SDS-3701

Compund	Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s/kg bw/day)	App. rate (kg/ha)	MAF	DDD (mg a.s/kg bw/day)	LD_{50} mix	TER
Chlorothalonil + SDS-3701	Cereals BBCH 30-39	Small omnivorous bird “lark”	12	0.75	1.0*	10.8	>1185	109.7
	Cereals BBCH ≥40		7.2		1.4	6.48		182.9
	Fruiting vegetables BBCH 71-89	Frugivorous bird "crow"	57.4	1.0	-	57.4		20.6
	Fruiting vegetables BBCH ≥50	Small granivorous bird “finch”	7.4			7.4		160.1
	Fruiting vegetables BBCH ≥50	Small omnivorous bird “lark”	7.2			7.2		164.6
	Fruiting vegetables BBCH 71-89	Frugivorous bird "starling"	49.4			49.4		24.0
	Fruiting vegetables BBCH ≥20	Small insectivorous bird “wagtail”	25.2			25.2		47.0

As shown in the table above, the Acute risk from the combination of chlorothalonil and the metabolite R128821 (SDS-3701) from the proposed uses is acceptable.

Long term

As the long term risk to birds could not be excluded in the screening step, a Tier 1 assessment is performed to assess the long term risk to birds from the proposed uses of ARY-0474-001 from the active substance chlorothalonil and the metabolite SDS-3701. The three possible proposed reproductive endpoints for chlorothalonil are presented to enable the expert discussion.

Table 9.2.2.2-4: Tier 1 assessment – Long term risk to birds from chlorothalonil

Compund	Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s/kg bw/day)	App. rate (kg/ha)	MAF	f_{TWA}	DDD (mg a.s/kg bw/day)	NOEL	TER
Chlorothalonil	Cereals BBCH 30-39	Small omnivorous bird “lark”	5.4	0.75	1.0*	0.53	2.1465	~~58.2~~	~~27.1~~
								119	55.4
								~~158~~	~~73.6~~
	Cereals BBCH ≥40		3.3		1.4		1.8365	~~58.2~~	~~31.7~~
								119	64.8
								~~158~~	~~86.0~~
	Fruiting vegetables BBCH 71-	Frugivorous bird "crow"	32.0	1.0	-		16.96	~~58.2~~	~~**3.4**~~
								119	7.0

Compund	Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s/kg bw/day)	App. rate (kg/ha)	MAF	f_{TWA}	DDD (mg a.s/kg bw/day)	NOEL	TER
	89							~~158~~	~~9.3~~
	Fruiting vegetables BBCH ≥50	Small granivorous bird “finch”	3.4				1.802	~~58.2~~	~~32.3~~
								119	66.0
								~~158~~	~~87.7~~
	Fruiting vegetables BBCH ≥50	Small omnivorous bird “lark”	3.3				1.749	~~58.2~~	~~33.3~~
								119	68.0
								~~158~~	~~90.3~~
	Fruiting vegetables BBCH 71-89	Frugivorous bird "starling"	20.7				10.97	~~58.2~~	~~5.3~~
								119	10.8
								~~158~~	~~14.4~~
	Fruiting vegetables BBCH ≥20	Small insectivorous bird “wagtail”	9.7				5.14	~~58.2~~	~~11.3~~
								119	23.1
								~~158~~	~~30.7~~

*according to the GAP only 1 application is possible before BBCH 40

Table 9.2.2.2-5: Tier 1 assessment – Long term risk to birds from SDS-3701

Compund	Crop grouping/ growth stage	Generic focal species	FIR/bw (mg food/kg bw/day)	Residue level (mg a.s./kg fresh weight)	MAF	f_{TWA}	DDD (mg a.s/kg bw/day)	NOEL	TER
0.53	Cereals BBCH 30-39	Small omnivorous bird “lark”	0.52	1.1	-	1[a]	0.49608	10.1	17.7
	Cereals BBCH ≥40								
	Fruiting vegetables BBCH 71-89	Frugivorous bird "crow"	0.93	0.02		0.53	0.88722		1024.5
	Fruiting vegetables BBCH ≥50	Small granivorous bird “finch”	0.28	1.1		1	0.26712		32.8
	Fruiting vegetables BBCH ≥50	Small omnivorous bird “lark”	0.52	1.1		1[a]	0.49608		17.7
	Fruiting vegetables BBCH 71-89	Frugivorous bird "starling"	1.62	0.02		0.53	1.54548		588.2
	Fruiting vegetables BBCH ≥20	Small insectivorous bird “wagtail”	0.79	1.1		1	0.75366		11.6

[a] As a conservative measure it is assumed that there is no residues decline in all food items, including the (25%) plant food items, where the residues decline of 10 days is considered appropriate. See not from the RMS on residues decline in food items for birds and mammals, above.

The Tier 1 assessment shows an acceptable risk to birds from use in cereals, but a remaining risk to crow in tomatoes if the most conservative endpoint is chosen (58.2 mg/kg bw/d). The refinements that were already rejected by the RMS, which were agreed upon by the MS/EFSA, have been crossed out. New proposed refinements of the notifers, and the opinion of the RMS on these refinements, have been added so that in the event that they are necessary they could be used to further refine the risk assessment.

Refined long-term risk assessment

~~**Risk to wagtail**~~

~~PT refinement~~

~~The notifier proposes refining the PT of the wagtail in tomatoes based upon a monitoring study in the UK:~~

~~*A generic field study was conducted in vegetable fields in England to determine a more realistic PT value for yellow wagtails (***Giessing & Wilkens, 2008***). PT values were calculated across all vegetables for 22 radiotracking sessions with 21 individual wagtails. A number of approaches have been used in this study to define the relevant wagtail population for calculation of PT. The most relevant approach for risk assessment purposes is considered to be the 'home range approach' i.e. all wagtails are included for which vegetables were within the home-range defined by the minimum convex polygon marked by radiotracking locations during a single day's radiotracking. These individuals are classed as "potential consumers" and EFSA (2009) recommends using data from both 'consumers' and 'potential consumers' to estimate PT. As a worst-case, the PT values have been used across all vegetables in this study. The mean PT is relevant for long-term risk assessment and therefore a* ***PT of 0.41*** *from 20 radiotracking sessions is applied. A 90th percentile PT value of* ***0.865*** *is also available. Both values will be used in the risk assessment.*~~

~~*It is considered that the number of individuals tracked within the study is relatively high, and that the number of crops used provides some certainty to the robustness of these data and therefore the 90th percentile is a conservative assessment and the mean PT value is more relevant to the higher tire refined risk assessment. The morphology from a birds eye view of the different crops involved in the study, (onion, carrots, lettuce, leeks and red beet), provide a suitable range to allow extrapolation of the crops in the study to other vegetables including the fruiting vegetables and pulses under this evaluation. It is also noted that these data from within this study were similar to those determined in the study on potatoes below, providing further evidence of the robust nature and the suitability to extrapolate between similar crops. It is considered that for these reasons the mean PT should be used. A UK assessment with the 90th percentile PT has also been included for country specific consideration.*~~

~~The RMS has previously evaluated the study and found it acceptable for use in risk assessment. The values for consumers (most relevant group according to EFSA (2009) in vegetable fields are shown in the table below.~~

~~**Table 9.2.2.2-05: PT values in vegetable fields according to Giessing & Wilkins, 2008**~~

Number, 90th percentile and mean PT of wood pigeon, skylark and yellow wagtail in several vegetable crops, based on 'consumer approach'.

		Yellow wagtail	
Crop	**N**	**90%tile [%]**	**Mean [%]**
Vegetables			
Onion	5	30.5	12.3
Leek	2	16.9	15.9
Carrot	4	70.1	36.9
Lettuce	13	44.6	21.6
Celery	10	44.1	17.8
Red beet	3	71.3	40.7
Radish	1	3.8	3.8
Total vegetable crop	19	86.5	43.4
Other crops			
Sugar beet	1	5.9	5.9
Potato	10	79.8	32.1
Cereal	8	39.2	12.4
Bean	0		
Drilled field	0		
Other (non-crop habitats)	21	74.2	40.2
Unknown (but no vegetable)	2	1.5	1.4

In principle, the RMS agrees that the study is well-conducted and useful for risk assessment, however, we note that the study was conducted in and around vegetable fields in the fenlands of Cambridgeshire (one trial site, Hasse Estate Farm (681 ha; carrot, onion, lettuce, leek and red beet)) and Norfolk (two trial sites: Rosedene Farm (1373 ha; carrot, onion, lettuce, leek, red beet, celery and radish) and Bars Hall Farm (396 ha; carrot, onion, lettuce and cabbage)) near the town of Littleport, Cambridgeshire, England. According to the document this region is a typical area of vegetable cultivation in Europe, however, it is not clear that this region is adequately representative of areas of vegetable cultivation in areas further from the UK. Further, although the RMS agrees that extrapolation from vegetable fields should be possible, we note that none of the vegetable types monitored were in the category of "fruiting vegetables". These areas of uncertainty will be carefully considered.Three focal bird species were defined as test organisms, the woodpigeon (*Columba palumbus*), skylark (*Alauda arvensis*) and yellow wagtail (*Motacilla [flava] flavissima*). During each tracking session a bird was tracked continuously from dawn till dusk, from first activity displayed in the morning until last activity performed in the evening, so that the location, habitat and behaviour could be recorded to get information of the home range, habitat selection and time budget of individuals. Every change in behaviour and location was accurately recorded every minute. Every tagged bird was tracked once, with the exception of five individuals who were tracked twice. The duplicate sessions of these birds were pooled for analysis. A tracking session was considered successful if it was possible to assign the location of the tracked individual properly for more than six hours. In order to describe the behaviour of the tracked bird as accurately as possible and to verify the location, optical devices were used. A safety distance of 30 m was kept in approaching the birds. This is an acceptably conservative methodology.

The study was carried out in the period between April 10 and July 10, 2007, however, the proposed application timing in tomatoes is from June onwards. However, an H-test (Kruskal-Wallis) did not show significant differences between the PT values for the various months. This means that there are no

~~obvious seasonal changes in the utilisation of vegetable fields by yellow wagtails during the study period.~~

~~Considering the uncertainty as to representativeness of the non-fruiting vegetables to fruiting vegetables, as well as other uncertainties associated with PT studies, as outlined in EFSA (2009), the RMS considers it appropriate to use the 90th percentile PT of **0.865** for risk assessment.~~

~~PD refinement~~

~~The notifier proposes refining the PD of wagtail as follows:~~

~~*The Birds of the Western Palearctic (Cramp, 2006[5]), which is probably the most complete reference work on European birds, includes the following statements on food of yellow wagtail:*~~

~~*"**Food**: Small invertebrates. 3 main foraging techniques (for more detailed breakdown, including use of high flight, see Wood 1976[6]). (1) Picking. Picks items from ground or water surface while walking. (2) Run-picking. Makes quick darting run at prey, picking it up either from surface or as it takes off. (3) Flycatching. Makes short flight from ground or perch, catching prey in mid-air – either in bill or by knocking it down with wings. (Smith 1950[7]; Davies 1977[8].) Occasionally takes insects from plants in hovering flight (Glutz von Blotzheim 1962[9]), or flies low over water snatching insects from surface (Kishchinski 1980[10]).*"~~

~~*The reference to occasionally taking insects from foliage indicates that this is not a frequent mode of foraging and hence that the assumption of 50% foliar: 50% ground insects over-estimates the proportion of foliar insects. Further evidence from the literature indicates that yellow wagtails avoided foraging in sugar beet fields and preferred to forage along tracks and ditch edges (Gilroy et al, 2009[11]). A further study of yellow wagtails in an agricultural landscape (Stiebel, 1997[12]) found that this species preferred areas of bare ground or sparse vegetation for foraging and that the most common way of feeding was picking prey from the ground, though some insects were caught in flight.*~~

~~*Therefore, the evidence is that the default diet assumption of 50:50 ground and foliar invertebrates in the diet is conservative and that the proportion of foliar insects will be much lower. This is demonstrated by a specific field study which examined the foraging behaviour of yellow wagtails in tomato fields (Miersch & Hahne, 2013). There is no reason to expect that yellow wagtail foraging techniques will differ significantly between crops and hence it is reasonable to consider the foraging data derived from the study on tomatoes for refining risk in all crops within this evaluation. The study in tomatoes showed that yellow wagtails take on average 70% of food from the ground and 30% from foliage and these proportions [should be] used in the risk assessment...*~~

~~The study summary was provided at a late stage and has not been evaluated by the RMS. Nonetheless, the RMS notes that the arguments presented by the notifier are contradicted by the~~

[5] Cramp, S (Ed), (2006) The Birds of the Western Palearctic on interactive DVD-ROM. Birdguides Ltd and Oxford Univ. Press.
[6] Wood J.B, (1976) The biology of yellow wagtails over-wintering in Nigeria. PhD thesis. Aberdeen.
[7] Smith S, (1950) The yellow wagtail. Collins, London.
[8] Davies N B (1977) Prey selection and social behaviour in wagtails. J. Anim. Ecol, 46: 37-57.
[9] Glutz von Blotzheim U.N, (1962) Die Brutvögel der Schweiz. Arau, Switzerland.
[10] Kischchinski A. A, (1980) Ptitsy Koryaksky nagor'ya (The birds of the Koryak Highland). Moscow.
[11] Gilroy et al (2009) Foraging habitat selection, diet and nestling condition in Yellow Wagtails *Motacilla flava* breeding on arable farmland. *Bird Study.* 56: 221-232.
[12] Stiebel (1997) Habitatwahl, Habitatnutzung und Bruterfolg der Schafstelze *Motacilla flava* in einer Agrarlandschaft. *Vogelwelt*. 118: 257-268.

study they referred to for refinement of the PT, where a PD via fecal analysis was also calculated for wagtails in vegetable fields. The remains in the faeces of the yellow wagtail contained invertebrates (99.0%), plant material (1.0%). The PD as proportion of dry weight calculated by applying the correction factors specific for yellow wagtail (correction factors for different families of arthropods and Poaceae seeds/leaves) is 99.8% for invertebrates and 0.2% for plant material. The PD for invertebrates can be subdivided into PD per habitat: 86.6% foliage (air), 1.7% ground and 11.5% foliage ground. Considering this, as well as the argumentation of the notifier, the PT will not be adjusted from the default 50% ground arthropods and 50% foliar arthroopods, as per the Guidance (2009).

A refined risk assessment for wagtails in tomatoes is shown in Table 9.2.2.2-06, below.

Table 9.2.2.2-06: Refined long-term risk to wagtail from use of chlorothalonil in tomatoes

Compund	Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s/kg bw/day)	App. rate (kg/ha)	MAF	PT	f_{TWA}	DDD (mg a.s/kg bw/day)	NOEL	TER
chlorothalonil	Fruiting vegetables BBCH ≥20	Small insectivorous bird "wagtail"	9.7	1	1	0.86	0.53	4.446965	14	**3.15**

As shown in Table 9.2.2.2-06, a risk to wagtail from the proposed use in tomatoes remains.

Risk to Frugivorous birds

PD

The applicant proposes adjusting the diet of starling, as shown here:

*According to Appendix A of the **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** the frugivorous birds 'starling' and 'crow' have to be considered for fruiting vegetables such as tomato at BBCH 71-89 (BBCH 71:' First fruit reaches typical size' at BBCH 89: 'Fully ripe: fruits have typical fully ripe colour'). Green tomatoes will not be eaten by birds because of their high content of solanine, a glykoalkaloid which is toxic with a bitter taste.*

Within the Tier I data in Appendix A of EFSA 2009, the diet of the starling is considered for this evaluation as 100% fruit. Within the CRD Bird Bible (Buxton et al. 1998), the diet of the starling is not considered to be 100% fruit. The starling (Sturnus vulgaris) feeds on a wide range of plant and animal material varying with season. Proportion of plant material in diet is less than 50% from April to June but between 50% and 95% during the remainder of year (Christensen et al. 1996[13]). In Poland during February-September 85% of food items were animal with almost no vegetable food items taken from March to June. Coleoptera (Carabidae and Scarabidaeidae among others) dominated animal fraction of diet (c. 40%) with Diptera and Hymenoptera making up c. 20% each. Lepidoptera were also found in reasonable numbers.

[13] Christensen, K.D., Falk, K. & Peterson, B.S. 1996. Feeding biology of Danish farmland birds. A literature study. Working Report No.12, Danish Environmental Protection Agency.

The "Bird Bible" (Buxton et al, 1998) provides a review of sources of information on the diet of the starling from the literature. Cultivated fruit are mentioned as part of the diet in two references; in Czechoslovakia 20% by number in adult diet was given as cultivated fruit whilst in another reference (Collinge, 1924-27[14]) cultivated fruit was given as 16% of the diet.

Considering that the proportion of plant material is given as less than 50% from April to June and nestling food is reported as almost entirely of animal origin, mainly insects (Christensen et al. 1996), it is highly unlikely that starlings will consume only fruit in the long-term during the breeding season. This is supported by the very high food intake rate of 1.62 times bodyweight indicated for a starling consuming only fruits given in Appendix A of EFSA 2009.

From these data, it is considered that the highest value for plant matter is 45% and for fruit specifically is 28%. Due to the variation in the data available a FIR/bw can be calculated for the mixture diet assuming 45% fruit as a worst case with the remaining food items (55%) as insects. It is considered that this covers the worst case fruit consumption and is therefore sufficiently conservative. The body weight of males from the CRD Bird Bible (Buxton et al. 1998) (84.7g for males) will also be used as a worst case using the CRD mixed diet calculator as shown in the tables below. Although the starling is a smaller bird, consideration of the diet of the crow needs to be considered to ensure the starling is still the worst case. According to the CRD Bird Bible (Buxton et al. 1998), the diet of the crow very rarely contains fruit or non-cereal grain plant material during the breeding season. In fact the highest amount of plant material which could be fruit is stated as 13% between May and August or 8 % between January and April. There are higher proportions of plant material in the diet available but these are between September and December when birds will not be breeding. Therefore it is considered that because of its higher body weight and lower consumption of fruit in its diet, the risk to the crow is covered by the assessment for the starling.

Table 10.1.1-19: Calculation of daily dietary consumption for the starling (84.7 g bodyweight)

Food type	**Moisture content** [a]	**Energetic content of food** [a b]	**Assimilation efficiency** [a]	**Energetic content of food, weighted by assimilation efficiency**	**Proportion of different food items in diet mix**	**Energy Uptake per gram of diet mix** [c]	**DEE**	**Daily food consumption** [d]
	(%)	**(kJ/g wet wt)**	**(%)**	**(kJ/g wet wt)**	**(% of diet wet wt)**	**(kJ/g wet wt)**	**(kJ)**	**(g wet wt/day)**
Fruit	*83.9*	*2.38*	*67*	*1.60*	*45*	*0.72*	-	*26.47*
Arthropods	*68.8*	*7.08*	*76*	*5.38*	*55*	*2.96*	-	*32.35*
Total	-	-	-	-	*100*	*3.68*	*216.4*	-

[a] *Taken from Appendix G, of EFSA 2009*

b incorporating moisture content

[c] *Calculated as Energetic content of food, weighted by assimilation efficiency x proportion of different food items in diet mix/100*

[d] *Calculated as (DEE ÷ Total energy uptake per gram of diet) x Proportion of different food items in diet mix*

[14] Collinge, W.E. (1924-27) The food of some British wild birds: a study in economic ornithology. 2nd edition. Published by the author, York.

The daily dietary consumption values have been normalised for body weight to give FIR/bw data for each species as shown below.

Table 10.1.1-20: Calculation of FIR/bw values for the starling consuming a mixed diet

Bird species	**Food type**	**Daily food consumption (g wet wt/day)**	**Body weight (g)**	**FIR/bw for specific food type (g fresh wt/g bw/day)**
Starling	*Fruit*	*26.47*	*84.7*	*0.31*
	Arthropods	*32.35*		*0.38*

The FIR/bw values for the respective food types can then be used to determine more realistic estimates of exposure and calculate refined TER values, based upon a starling consuming a mixed diet. Since the starling feeds on invertebrate food taken on soil surface or just below soil surface by bill-probing (Christensen et al. 1996), it will be a reasonable worst-case assumption to assume that the invertebrate food component has the same residue as ground insects. Since fruits are present from BBCH 71, it is appropriate to use the mean RUD of 3.5 for ground-dwelling invertebrates. The refined MAF and ftwa for arthropods calculated above has also been used for the arthropod proportion of the diet.

Table 10.1.1-21: Refined long-term risk (TER_{LT}) to starling feeding on a mixed diet

Crop grouping / growth stage	**Food type**	**RUD (mg a.s./kg)**	**App. rate (kg a.s./ha)**	**FIR/bw**	**MAF**	**ftwa**	**Refined DDD (mg a.s/kg bw/day)**	**NOEL (mg/kg bw/day)**	**TER_{LT}**
Fruiting vegetables (1 x 1000 g/ha) BBCH 71-89	*Fruit (tomato)* [a]	*12.8*	*1.0*	*0.31*	*-*	*0.53*	*2.10*	*16*	*-*
	Arthropods [a]	*3.5*		*0.38*	*-*	*0.20*	*0.266*		*-*
	Total	*-*		*-*	*-*	*-*	*2.37*		*6.8*

[a] *Taken from Appendix F, of EFSA 2009*

*Values in **bold** fall below the trigger*

When the refined FIR/bw values are compared to the NOEL of 16 mg a.s./kg bw/d the resulting TER_{LT} value is above the Tier I trigger of 5 indicating an acceptable risk to frugivorous birds in tomatoes following use of A14111B according to the proposed use pattern.

Since the RMS is not able to check whether the referenced studies of starling diet were performed appropriately, nor the robustness of the data and uncertainties involved, the RMS does not accept a quantitative refinement of the starling diet as proposed by the notiifer. Nonetheless, the RMS agrees that the starling diet is unlikely to be 100% fruit (tomatoes). This will be considered in a weight of evidence approach.

Residues

Further, the notifier proposes refining the residues on tomatoes, as follows:

In addition, measured residue data on field tomatoes are reported in ***MCP Section 6****. Azoxystrobin and chlorothalonil were applied to field tomato as A14111B. Two applications separated by a 6-7 day interval were made at 200 g a.s./ha for azoxystrobin and 1000 g a.s./ha for chlorothalonil. Residues on treated tomato whole fruit specimens taken immediately after the last application (0 DALA) can be used to refine the RUD. Measured residues for chlorothalonil as reported in are summarised in the table below. Further residue trials are ongoing and additional data is available within the current* ***Part B, Section 4 at*** *3 DALA which demonstrate the decline in residues over time, supporting use of refined residues in this assessment.*

Table 10.1.1-22: Measured residues of chlorothalonil on field tomatoes

Trial / Sample No.	***Application rate (kg a.s./ha)***	***Number of applications***	***Sampling interval (days)***	***Crop Part***	***Chlorothalonil residue (mg/kg)***
S11-00520-01 / 001	*1.0*	*2*	*0 DALA*	*Whole fruit*	*1.6*
S11-00520-03 / 001					*0.23*
S11-00520-04 / 001					*3.0*
S11-00520-05 / 001					*6.4*
S11-00521-02 / 001					*2.2*
S11-00521-03 / 001					*1.1*
S11-00521-04 / 001					*2.2*
Mean					***2.39***

Long-term risk assessment typically used the mean RUD which here would be 2.39 mg a.s./kg, however to ensure conservatism, the highest RUD of 6.41 has been used in this case. Using worst case residue concentrations from the data presented in Table 10.1.1-20 represents an extremely conservative approach given that this results from 2 applications.

Table 10.1.1-23: Refined long-term risk (TER_{LT}) to starling feeding on a mixed diet considering worst case residues on tomatoes

Crop grouping / growth stage	***Food type***	***Refined RUD (mg a.s./kg)***	***App. rate (kg a.s./ha)***	***FIR/bw***	***MAF***	***ftwa***	***Refined DDD (mg a.s/kg bw/day)***	***NOEL (mg/kg bw/day)***	***TER_{LT}***
Fruiting	*Fruit*	*6.41*	*1.0*	*0.31*	*-*	*0.53*	*1.05*	*16*	*-*

~~Crop grouping / growth stage~~	~~Food type~~	~~Refined RUD (mg a.s./kg)~~	~~App. rate (kg a.s./ha)~~	~~FIR/bw~~	~~MAF~~	~~ftwa~~	~~Refined DDD (mg a.s/kg bw/day)~~	~~NOEL (mg/kg bw/day)~~	~~TER_{LT}~~
~~vegetables (1 x 1000 g/ha) BBCH 71-89~~	~~(tomato)~~ [a]								
	~~Arthropods~~ [b]	~~3.5~~		~~0.38~~	~~-~~	~~0.20~~	~~0.266~~		~~-~~
	~~Total~~	~~-~~		~~-~~	~~-~~	~~-~~	~~1.32~~		~~12~~

[a] ~~Refined worst case RUD using actual residue data~~

[b] ~~Taken from Appendix F, of EFSA 2009~~

~~*When considering conservative measured residues on tomatoes, the refined TER_{LT} values for chlorothalonil are greater than the Tier I trigger of 5 indicating an acceptable risk to frugivorous birds in fruiting vegetables following use of A14111B according to the proposed use pattern.*~~

~~The EFSA Guidance (2009) advises that sufficient evidence must be provided before a RUD can be adjusted, as a large number of trials were used to set the default RUDs in the Guidance. The notifier has not provided evidence as to why the RUDs for tomatoes should be adjusted from the default RUDs available in the Guidance, particularly where starling is concerned, as the default RUDs in the Guidance are based upon tomatoes. Unless a better argumentation is provided, the RMS does not accept a refinement of the RUD on tomatoes.~~

~~Considering the above, there are no refinements available for starling or crow, and the risk to frugivorous birds from the proposed use of chlorothalonil in tomatoes remains. The risk to insectivorous birds from the proposed use of chlorothalonil in tomatoes is also unacceptable.~~

Refinement of the risk to large frugivorous bird, crow

To address any remaining risk to large frugivorous bird, crow, from chlorothalonil, the notifiers propose using an adjusted shortcut value in the Tier 1 risk assessment, to reflect the default residues in tomatoes, rather than the residues in gourds from Baril et. al. (2005), which are used as the default residue for crow in fruiting vegetables according to EFSA (2009). The RMS agrees that the default residues from tomatoes are more appropriate for the requested GAP, where only use in tomato is requested. Therefore, the refined shortcut value for crow would be RUDmean x FIR/bw = 12.8 x 0.93 = 11.9. The refined risk assessment considering an SV of 11.9 for crow is shown below in Table 9.2.2.2-6.

Table 9.2.2.2-6: Refined assessment – Long term risk to birds from chlorothalonil

Compund	Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s/kg bw/day)	App. rate (kg/ha)	MAF	f_{TWA}	DDD (mg a.s/kg bw/day)	NOEL	TER
Chlorothalonil	Fruiting vegetables BBCH 71-	Frugivorous bird "crow"	11.9	1.0	-		6.307	~~58.2~~	~~9.2~~
								119	18.9

Compund	Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s/kg bw/day)	App. rate (kg/ha)	MAF	f_{TWA}	DDD (mg a.s/kg bw/day)	NOEL	TER
	89							~~158~~	~~25.1~~

In case further refinement is necessary for chlorothalonil, the notifiers also refer to the study of Schmidt, et. al. (2009). The RMS has evaluated this study, which can be found above in Section B.9.1.3. The RMS considers the DT_{50} calculated from residues in *Zophobas* larvae (1.6 days) from this study to be acceptable. However, it should be further considered whether a DT_{50} calculated from residues decline in one species under non-field conditions is acceptable for use in the risk assessment, considering the uncertainties in residues decline in arthropods. The UK has proposed a "worst-case" value of 3.1 days, based on the maximum DT_{50} for crickets, although the data in crickets was highly variable. The notifiers also propose using the DT_{50} of 3.1 days to refine the residues decline in arthropod food items.

The notifiers also refer to the study of Miersch and Hahne (2014), which the RMS has evaluated (see Section 9.1.1.1). the RMS does not consider this study reliable enough to be used in the risk assessment, as large uncertainties exist as to whether the methodology was appropriate to calculate the diet of wagtails. Furthermore, the notifier has not presented information to support the use of wagtail as focal species for ecological refinements.

The notifier also proposes PD adjustments for frugivorous birds starling and crow, based on information from the CRD Bird Bible, however, again, no focal species data is presented to support the use of these species for ecological refinements. Thus, the RMS does not consider these potential refinements acceptable.

B.9.2.2.3 Acute dietary risk to mammals

Note: The risk assessment presented below has been adjusted according to the proposals above regarding endpoints and metabolite exposure. These will be discussed in an expert meeting, but reflect the RMS opinions.

Screening step

The acute 'daily dietary dose' (DDD) is calculated by multiplying the Shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha.

$$DDD_{multiple\ applications} = \text{application rate (kg a.s./ha)} \times SV$$

Table 9.2.2.3-1: Screening step – Acute risk to mammals from A14111B

Compound	Crop group	Indicator species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg/ha)	MAF	DDD (mg a.s./kg bw/ day)	LD_{50}	TER
Chlorothalonil	Cereals	Small herbivorous mammal	118.4	0.75	1.2	106.56	> 5000	46.92
	Fruiting vegetables		136.4	1.0	-	136.4		36.66

Chlorothalonil/ azoxystrobin	Cereals		118.4	0.90	1.2	127.872	3045	23.81
	Fruiting vegetables		136.4	1.2	-	163.68		18.60

Table 9.2.2.3-2: Screening step – acute risk to mammals from exposure to SDS-3701

Compound	Crop group	Indicator species	FIR/bw (mg food/kg bw/day)	Residue level (mg/kg fresh weight)	MAF	DDD (mg a.s./kg bw/ day)	LD_{50}	TER
SDS-3701	Cereals	Small herbivorous mammal	1.68	1.1	-	1.848	242	131.0
	Fruiting vegetables		1.33			1.463		165.4

Table 9.2.2.1-3: Screening step – acute risk to mammals from exposure to chlorothalonil + SDS-3701

Compound	Crop group	Indicator species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg/ha)	MAF	DDD (mg a.s./kg bw/ day)	LD_{50}	TER
Chlorothalonil	Cereals	Small omnivorous bird	158.8	0.75	1.2	143	> 2367.8	18.5
	Fruiting vegetables			1.0	-	159		14.5

As shown in the tables above, all uses pass in the acute screening step for dietary exposure.

B.9.2.2.4 Long term dietary risk to mammals

Screening step

The long term 'daily dietary dose' (DDD) is calculated by multiplying the Shortcut value (SV) based on the mean percentile residues by the application rate in kg a.s./ha and a default f_{TWA} of 0.53 taking into account a default residue decline (DT_{50}) of 10 days. In the event of multiple applications, a multiple application factor (MAF) is also included.

$$DDD_{multiple\ applications} = \text{application rate (kg a.s./ha)} \times SV \times 0.53 \times (MAF)$$

Table 9.2.2.4-1: Screening step – Long term risk to mammals from chlorothalonil

Compound	Crop group	Indicator species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg/ha)	f_{TWA}	MAF	DDD (mg a.s./kg bw/ day)	NOEL	TER
Chlorothalonil	Cereals	Small herbivorous mammal	48.3	0.75	0.53	1.4	26.87895	22.6	**0.84**
	Fruiting vegetables		72.3	1.0		-	38.319		**0.59**

Table 9.2.2.4-2: Screening step – Long term risk to mammals from exposure to SDS-3701

Compound	Crop group	Indicator species	FIR/bw (mg food/kg bw/day)	Residues (mg/kg fresh weight)	f_{TWA}	MAF	DDD (mg a.s./kg bw/ day)	NOEL	TER
SDS-3701	Cereals	Small	1.68	1.1	0.53	-	0.97944	1.5	**1.5**

	Fruiting vegetables	herbivorous mammal	1.33				0.77539		**1.9**

As shown in the tables above, both chlorothalonil and the metabolite SDS-3701 should be further assessed at Tier 1.

Tier 1 assessment

As the long term risk to mammals could not be excluded in the screening step, a Tier 1 assessment is performed to assess the long term risk to mammals from the proposed uses of A14111B from the active substance chlorothalonil and the metabolite SDS-3701.

Table 9.2.2.4-3: Tier 1 assessment – Long term risk to mammals from chlorothalonil

Compund	Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s/kg bw/day)	App. rate (kg/ha)	MAF	f_{TWA}	DDD (mg a.s/kg bw/day)	NOEL	TER
Chlorothalonil	Cereals BBCH 30-39	Small omnivorous mammal "mouse"	3.9	0.75	1.0[a]	0.53	2.17035	22.6	10.41
	Cereals BBCH ≥40		2.3		1.4		1.27995		17.66
	Cereals BBCH ≥40	Small herbivorous mammal "vole"	21.7		1.4		12.07605		**1.87**
	Cereals BBCH ≥20	Small insectivorous mammal "shrew"	1.9				1.05735		21.37
	Fruiting vegetables BBCH 71-89	Frugivorous mammal "rat"	25.2	1.0	-		13.356		**1.69**
	Fruiting vegetables BBCH ≥50	Small omnivorous mammal mouse"	2.3				1.219		18.54
	Fruiting vegetables BBCH ≥50	Small herbivorous mammal "vole"	21.7				11.501		**1.97**
	Fruiting vegetables BBCH ≥20	Small insectivorous mammal "shrew"	1.9				1.007		22.44

[a] The GAP indicates only one application is possible before BBCH 40

Table 9.2.2.4-4: Tier 1 assessment – Long term risk to mammals from SDS-3701

Compund	Crop grouping/ growth stage	Generic focal species	FIR/bw (mg/kg bw/day)	Maximum residues (mg/kg fresh weight)	f_{TWA}	DDD (mg a.s/kg bw/day)	NOEL	TER

Compund	Crop grouping/ growth stage	Generic focal species	FIR/bw (mg/kg bw/day)	Maximum residues (mg/kg fresh weight)	f_{TWA}	DDD (mg a.s/kg bw/day)	NOEL	TER
SDS-3701	Cereals BBCH 30-39	Small omnivorous mammal "mouse"	0.27	1.1	1[a]	0.25758	1.5	5.1
	Cereals BBCH ≥40							
	Cereals BBCH ≥40	Small herbivorous mammal "vole"	1.33		0.53	1.26882		**1.9**
	Cereals BBCH ≥20	Small insectivorous mammal "shrew"	0.55		1[a]	0.5247		**2.5**
	Fruiting vegetables BBCH 71-89	Frugivorous mammal "rat"	0.73	0.02	0.53	0.007738		193.8
	Fruiting vegetables BBCH ≥50	Small omnivorous mammal mouse"	0.27	1.1	1[a]	0.25758		5.1
	Fruiting vegetables BBCH ≥50	Small herbivorous mammal "vole"	1.33		0.53	1.26882		**1.9**
	Fruiting vegetables BBCH ≥20	Small insectivorous mammal "shrew"	0.55		1[a]	0.5247		**2.5**

[a]No residues decline is assumed for non-plant food items as a conservative measure. This is particularly conservative in the case of omnivorous mammal, where 25% of the diet is plant-based and would show quick residues decline.

As shown in the tables above, the risk to small herbivorous mammals from the use in cereals remains for both chlorothalonil and the metabolite SDS-3701, and the risk to small herbivorous and frugivorous mammals from the use in tomatoes remains for chlorothalonil and small herbivorous and small insectivorous mammals from the metabolite SDS-3701. A refined risk assessment is required for all proposed uses.

Refined long-term risk assessment

Since the notifier proposed a higher chronic endpoint for chlorothalonil than was used by the RMS, no refined risk assessment was presented for the risk to frugivorous and herbivorous mammals from the proposed use in tomatoes, nor to the small herbivorous mammal from the use in cereals. However, some of the refinements proposed by the notifier to address the risk from SDS-3701 can also be applied to the risk from chlorothalonil.

Vole

The notifier proposes the following refinements for the risk to small herbivorous mammals from the metabolite, SDS-3701:

The small herbivorous mammal 'vole' is considered to consume non crop grasses and non-grass herbs. As this evaluation is for a plant metabolite, the residue of SDS-3701 in grasses and non-grass

herbs below the crop canopy will be affected by deposition in the same way that residues of applied chlorothalonil will. Therefore it is considered appropriate to refine the risk to voles using interception. Within Appendix E of the EFSA Guidance on Bird and Mammal Risk Assessment on 'Impact of crop interception on residues on plant food items', in referring to deposition estimates for Tier I, states that 'The deposition factors provided for the different crops and growth stages are likely to reflect conservative estimates. In the context of a higher-tier assessment, the more detailed values of FOCUS Groundwater guidance report (FOCUS, 2000[15]) may therefore also be used'. Therefore, this risk assessment will be refined using FOCUS Groundwater guidance interception values.
According to FOCUS Groundwater guidance, for cereal growth stages relevant to the occurrence of voles in cereals of >BBCH 40, the crop interception is typically 90% (FOCUS, 2000).
According to FOCUS Groundwater guidance, for tomatoes relevant to the occurrence of voles at BBCH 40-89, the crop interception is typically 80% (FOCUS, 2000).

Table 10.1.2-20: Refined RUD for small herbivorous mammals in cereals

Crop grouping	***Tier I deposition factor***	***FOCUS gw deposition factor***	***RUD (mg/kg)***	***Refined RUD (mg/kg)***
Cereals BBCH > 40 (2 x 750 g/ha)	*0.3*	*0.1*	*1*	*0.1*
Fruiting vegetables BBCH > 50	*0.3*	*0.2*	*1*	*0.2*

The refined RUD can then be used to determine a more realistic estimates of exposure and to calculate a refined TER value. This is shown in the table below.

Table 10.1.2-21: Refined assessment - long-term risk (TER_{LT}) to small herbivorous mammals from SDS-3701 (NOEL = 1.5 mg a.s./kg bw/d)

Crop grouping / growth stage	***Generic focal species***	***Refined RUD (mg/kg)***	***FIR/bw***	***ftwa***	***Refined DDD (mg/kg bw/day)***	***TER_{LT}***
Cereals BBCH > 40	*Small herbivorous Mammal 'vole'*	*0.1*	*1.33*	*0.53*	*0.0705*	*21*
Fruiting vegetables BBCH > 50		*0.2*			*0.141*	*11*

When the refined DDDs for SDS-3701 are compared to the NOEL of 1.5 mg a.s./kg bw/d the resulting TER_{LT} is above the trigger and no further consideration is required.
The RMS agrees that the interception for both cereals and tomatoes can be further refined at the late growth stages (according to appendix E: ≥ BBCH 30 for cereals and ≥ BBCH 51 for solanaceous fruiting vegetables), and that these refinements can be applied to both chlorothalonil and the plant metabolite SDS-3701. The values presented by the notifier are appropriate according to FOCUS,

[15] FOCUS (2000): FOCUS Groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater scenarios workgroup, EC Document Reference SANCO/321/2000 rev. 2, 2002 pp; in conjunction with: Generic guidance for

2014. The RMS considers this to be acceptable. The risk assessment presented by the notifier, however, is not acceptable, as the residue value of 1 mg/kg for SDS-3701 was not accepted by the RMS.

The SV for voles in cereals (BBCH ≥ 40) and fruiting vegetables (BBCH ≥ 50) in Tier 1 is based upon the RUD*FIR/bw*interception and is equal to 54.2*1.33*0.3 = 21.7. The refined deposition factor can therefore be calculated by re-calculating the SV as 54.2*1.33*0.1 = 7.2 for cereals; and 54.2*1.33*0.2 = 14.4 for tomatoes. The results using this value in the refined risk assessment for chlorothalonil and SDS-3701 in cereals are shown in Tables 9.2.3.2-5 and 9.2.3.2-6.

Table 9.2.3.2-5: Refined long term risk to vole from use of chlorothalonil in cereals (BBCH 40-69) and tomatoes (BBCH 51-89)

	AR (kg a.s./ha)	SV*	MAF	f_{TWA}	DDD	NOEL (mg/kg bw/d)	TER
Cereals (2 x 750 g/ha, 14 d interval)							
small herbivorous mammal "vole"	0.75	7.2	1.4	0.53	4.0068	22.6	5.64
Tomatoes (1 x 1000 g/ha)							
small herbivorous mammal "vole"	1	14.4	1	0.53	3.816	22.6	**2.96**

Table 9.2.3.2-6: Refined long term risk to vole from exposure to SDS-3701 in cereals (BBCH 40-69) and tomatoes (BBCH 51-89)

	Residues mg metabolite /kg	FIR/bw	Interception	MAF	f_{TWA}	DDD	NOEL (mg/kg bw/d)	TER
Cereals (2 x 750 g/ha, 14 d interval)								
small herbivorous mammal "vole"	1.1	1.33	0.1	1	0.53	0.126882	1.5	19.3
Tomatoes (1 x 1000 g/ha)								
small herbivorous mammal "vole"	1.1	1.33	0.2	1	0.53	0.253764	1.5	9.7

As shown in the Tables above, the risk to small herbivorous mammal from chlorothalonil in both cereals is acceptable, but not in tomatoes. The long term risk to small herbivorous mammal from the metabolite is acceptable.

Frugivorous mammal

To refine the remaining risk to frugivorous mammal from the use of chlorothalonil in tomato, the notifier proposes that the default residues value for tomatoes from EFSA (2009) be used rather than the default residues value from gourds. Since the proposed application is only in tomatoes, the RMS agrees with this proposed refinement. Therefore, the refined shortcut value for large frugivorous mammal would be RUDmean x FIR/bw = 12.8 x 0.73 = 9.3. The refined risk assessment considering an SV of 9.3 for frugivorous mammal is shown below in Table 9.2.2.2-5.

Table 9.2.3.2-7: Refined assessment – Long term risk to mammals from chlorothalonil

Compund	Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s/kg bw/day)	App. rate (kg/ha)	MAF	f_{TWA}	DDD (mg a.s/kg bw/day)	NOEL	TER
Chlorothalonil	Fruiting vegetables BBCH 71-89	Frugivorous bird "rat"	9.3	1.0	-	0.53	4.95	22.6	**4.6**

The notifier further argues that it is not possible for the brown rat (used as the default species in EFSA, 2009) to consume its entire diet from tomatoes, and therefore proposes a diet of 50% tomato and 50% cereal seeds and calculate a new FIR/bw to refine the risk assessment. Whilst the RMS agrees that the rat is unlikely to consume a diet of only tomatoes, we are also reluctant to accept a "hypothetical" diet in its place. The notifier has not presented data to address the actual focal species in tomato, nor to support the proposed PD. Thus, the RMS does not consider this refinement acceptable.

Insectivorous mammal

To refine the remaining risk to small insectivorous mammal from the metabolite R182281 (SDS-3701) the notifier proposes an interception value be used, as the residues value of 1.1 mg/kg fresh weight does not include any interception, and the small insectivorous mammal is assumed to eat 100% ground arthropods. They refer to FOCUS (2000) groundwater values to propose a crop interception value of 80% in both cereals and tomato, resulting in a refined residue level of 0.22.

The RMS agrees that the interception value for tomatoes at BBCH 50-89 is 80%, according to FOCUS (2015). For spring and winter cereals, FOCUS (2015) give an interception value of 80% at BBCH 30-39 and 90% at 40 – 69. Thus, an interception value of 80% is appropriate for food items on the ground. However, considering the fact that the residues value used is a surrogate in any case, the RMS would prefer to use the highest soil PEC of 0.351 mg/kg soil dw as a more appropriate surrogate for the ground dwelling arthropods in question. A refined risk assessment using this value in the risk assessment is shown below.

Table 9.2.3.2-8: Refined long term risk to insectivorous mammal from exposure to SDS-3701 in cereals

	Residues mg metabolite /kg	FIR/bw	MAF	f_{TWA}	DDD	NOEL (mg/kg bw/d)	TER
Cereals (2 x 750 g/ha, 14 d interval)							
Small insectivorous mammal "shrew"	0.351		1	1	0.19305	1.5	7.8
Tomatoes (1 x 1000 g/ha)							
Small insectivorous mammal "shrew"	0.351		1	1	0.19305	1.5	7.8

The risk to small insectivorous mammal from the metabolite can be considered acceptable.

In summary, the risk to mammals from the proposed use in cereals is acceptable. The risk to large frugivorous mammal from the proposed use in tomato is not acceptable, thus, the use in tomatoes is not considered a safe use.

The notifier submitted a focal species study in tomato in Italy, which suggested that voles were less relevant in tomatoes in Italy, however, an even smaller species of small mammal, microtus minutus, was found. Harvest mice are known to be both herbivorous and granivorous, and have a lower bodyweight than the common vole, thus, this species might be considered relevant. Considering this, and the fact that any grains in the diet in tomato fields would likely be weed seeds and have similar or less interception than grass and weeds, the RMS considers the vole to be an appropriate focal species in tomato to cover the sensitive groups. In addition, the study has been performed in Italy, and its relevance in other areas of Europe is unclear.

B.9.2.3 Drinking water risk assessment for birds and mammals

In line with EFSA's Bird and Mammal Guidance Document (2009), the risk to birds and mammals through drinking contaminated water has been assessed. The 'puddle scenario' is considered relevant for the proposed uses of A14111B. This relates to birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop.

B.9.2.3.1 Screening step

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

Chlorothalonil has a Koc of 1288 L/kg (mean), therefore, the trigger value is 3000, however, SDS-3701 has a Koc of 395.3 L/kg (mean) of , therefore, the trigger value is 50. The ratios of effective application rate to relevant endpoints are presented in the following table.

Table B.9.2.4-01 Drinking water assessment – screening step for birds for the proposed uses of A14111B

Time scale	Max. application rate	MAF	Effective application rate	Endpoint	Ratio	Trigger value
Chlorothalonil						
Cereals (2 x 0.075 kg a.s./ha, min. interval of 14 days)						
Acute	750 g a.s./ha	1.71 [a]	841.4 g a.s./ha	LD_{50} = >2000 mg a.s./kg bw	0.64	3000
Long-term				NOEL = 14 mg a.s./kg bw/d	91.6	
Tomatoes (1 x 1.0 kg a.s./ha)						
Acute	1000 g a.s./ha	1.0	1000 g a.s./ha	LD_{50} = >2000 mg a.s./kg bw	0.50	3000
Long-term				NOEL = 14 mg a.s./kg bw/d	71.4	
SDS-3701						
Cereals (2 x 0.075 kg a.s./ha, min. interval of 14 days)						
Acute	223 g	1.98[a]	442a.s./ha	LD_{50} = 158 mg a.s./kg bw	2.8	50

Long-term	a.s./ha[b]			NOEL = 7 mg a.s./kg bw/d	**63.1**	
Tomatoes (1 x 1.0 kg a.s./ha)						
Acute	298 g a.s./ha[b]	1.0	298 g a.s./ha	LD_{50} = 158 mg a.s./kg bw	1.88	50
Long-term				NOEL = 7 mg a.s./kg bw/d	42.5	

[a] Calculated in line with Section 5.5 of EFSA (2009), based on a worst-case, non-normalized soil DT_{50} of 28.4 for chlorothalonil and a DT_{50} of 609 for SDS-3701, see Section CA 8.
[b] calculated assuming max 32% formation in soil and correcting for mass (thus: AR*0.32*0.93)

The above ratios for chlorothalonil are below the trigger value of 3000 indicating that no further assessment of the risk to birds from drinking water is required. However, the value for long term exposure to the metabolite SDS-3701 from use in tomatoes is above the trigger of 50, therefore a drinking water assessment for long term risk to birds should be carried out for the metabolite.

Table B.9.2.4-02 Drinking water assessment screening step for mammals for the proposed uses of A14111B

Time scale	Max. application rate	MAF	Effective application rate	Endpoint	Ratio	Trigger value
Chlorothalonil						
Cereals (2 x 0.075 kg a.s./ha, min. interval of 14 days)						
Acute	750 g a.s./ha	1.71 [a]	841.4 g a.s./ha	LD_{50} = >5000 mg a.s./kg bw	0.27	3000
Long-term				NOEL = 22.6 mg a.s./kg bw/d	56.7	
Tomatoes (1 x 1.0 kg a.s./ha)						
Acute	1000 g a.s./ha	1.0	1000 g a.s./ha	LD_{50} = >5000 mg a.s./kg bw	0.2	3000
Long-term				NOEL = 22.6 mg a.s./kg bw/d	44.2	
SDS-3701						
Cereals (2 x 0.075 kg a.s./ha, min. interval of 14 days)						
Acute	223 g a.s./ha[b]	1.98[a]	442 g a.s./ha	LD_{50} = 242 mg a.s./kg bw	1.83	50
Long-term				NOEL = 1.5 mg a.s./kg bw/d	**294.6**	
Tomatoes (1 x 1.0 kg a.s./ha)						
Acute	298 g a.s./ha[b]	1.0	298 g a.s./ha	LD_{50} = 242 mg a.s./kg bw	1.23	50
Long-term				NOEL = 1.5 mg a.s./kg bw/d	**198.4**	

[a] Calculated in line with Section 5.5 of EFSA (2009), based on a worst-case, non-normalized soil DT_{50} of 28.4 for chlorothalonil and a DT_{50} of 609 for SDS-3701, see Section CA 8.
[b] calculated assuming max 32% formation in soil and correcting for mass (thus: AR*0.32*0.93)

The above ratios for chlorothalonil are below the trigger value of 3000 indicating that no further assessment of the risk to mammals from drinking water is required. However, the value for long term exposure to the metabolite SDS-3701 from use in tomatoes is above the trigger of 50, therefore a drinking water assessment for long term risk to mammals should be carried out for the metabolite.

B.9.2.3.2 Tier 1

The assessment is performed considering the worst-case use in cereals and according to EFSA (2009). The PECpuddle is calculated considering the AReff as presented in the table above and the Koc of 395.3 for SDS-3701.

Table B.9.4.2-01: Tier 1 drinking water assessment for long term risk to birds and mammals from SDS-3701

PEC_{puddle}	DWR (L/kg bw/d)	DDD	NOEL	TER	Trigger
Birds					

0.0721	0.46	0.033166	7	211.06	5
Mammals					
0.0721	0.24	0.017304	1.5	86.69	5

As shown in the table above, the long term risk to birds and mammals from SDS-3701 in drinking water is considered acceptable.

B.9.2.4 Effects of secondary poisoning

According to **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**, substances with a log P_{OW} greater than 3 have potential for bioaccumulation. Chlorothalonil has a log P_{OW} value of 2.94. Consequently, it does not pose an unacceptable risk of secondary poisoning and further assessment is not required. For the chlorothalonil metabolite R182281 (SDS-3701), the estimated log P_{OW} for the un-dissociated (neutral) form is 3.55. However, R182281 is a strong acid with a pKa value of 0.7, at environmentally relevant pHs the P_{OW} of R182281 is approximately 0.01 (log P_{OW} = -2.0) with negligible bioaccumulation potential.

B.9.2.4.1 Biomagnification in Terrestrial Food Chains

For chlorothalonil the results from adsorption, distribution, metabolism and excretion (ADME) studies did not indicate a potential for accumulation, as the tissue residues 7 days after application were always <1% of applied dose (refer to the **Review Report for Chlorothalonil *SANCO/4343/2000 final (revised) 28. September 2006***).

B.9.2.5 Endocrine disruption

The mammalian toxicology package does not seem to indicate direct effects of chlorothalonil on the estrogen, androgen or thyroid pathways of the endocrine system in mammals. The bird reproduction studies show reduced egg production, but it is not possible to determine whether this was a result of any direct effect on estrogen pathways. The endpoint used in the reproductive risk assessment above is based upon egg parameters and therefore is assumed to be protective. Effects on the thyroid of terrestrial amphibians cannot be excluded (see aquatic risk assessment and section 9.4.6 for more information).

B.9.2.6 Conclusions

The above risk assessment showed the following:

- The proposed use in cereals is considered acceptable for birds and mammals.
- The proposed use in tomatoes still shows unacceptable risk to the small herbivorous mammal and the large frugivorous mammal, rat, though it may be noted that the diet of 100% tomatoes is considered quite conservative.

B.9.3 Effects on aquatic organisms

B.9.3.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Report:	IIIA, 10.2.1/01 (numbering of Volume 2 original DAR). Wütrich, V., 1990. Daconil 2787[7] Extra: 96-hour acute toxicity study (LC_{50}) in the rainbow trout. Generated by: RCC Umweltchemie AG Submitted by: Zeneca Report No.: 258052 Date: February 7, 1990 GLP, Unpublished
Previous evaluation	In DAR (2000) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	mean weight [g]	mean length [cm]	Dura-tion	test method	water hard-ness*	pH	T [°C]	Crite-rion	Value	Unit	a/n	Ri
Daconil 2878 Extra	Oncorhynchus mykiss	2.9	6.5	96 h	static	238	7.9-8.4	14	LC50	0.20	mg/l	n	2

* in mg/l as $CaCO_3$

Description

Daconil 2787 Extra is a suspension concentrate containing 40.4% chlorothalonil. Five test concentrations, 0.095 - 1.0 mg/l, plus control. Ten fish per vessel. Actual concentrations chlorothalonil measured at 0, 2, 48, and 96 hours at 0.095 and 1.0 mg/l (with and without fish), and at 0 and 2 hours at 0.308 and 1.0 mg/l (both with fish) by GC with electron capture detection (recovery 89.7%). Test according to OECD203.

Results

In the 1.0 mg/l vessel without fish the actual concentration decreased from 84% at t=0 to 58% of nominal at t=96h. In the 0.095 mg/l vessel the actual concentration decreased from 100 to 9% at 48 hours and below the detection limit at 96 hours. The two remaining samples decreased from 90-98% to 73-80% in two hours. Based on nominal concentrations the 96-hours LC50 0.195 mg/l (95% confidence interval 0.160-0.259 mg/l) calculated using logit method.

Remarks

As not all concentrations were measured, the measured concentrations cannot be used for calculation of the LC50. The results in the heading table are recalculated with the trimmed Spearman-Karber method, based on Hamilton et al (1977/78): 95% confidence interval 0.18-0.24 mg/l. The incipient LC50 is probably reached. The result 96h LC50 0.20 mg/l is not used for risk evaluation, because it is based on nominal concentrations, which is not acceptable as concentrations were not maintained at >80% of nominal.

Report:	IIIA, 10.2.1/02 (numbering of Volume 2 original DAR). Gelin, M. D., Laveglia, J., Machado, M. W., 1992. BRAVO[7] 720 - Acute toxicity to bluegill sunfish (*Lepomis macrochirus*) under flow-through conditions. Generated by: Ricerca, Inc. and Springborn Laboratories Inc. Submitted by: Zeneca Report No.: 5088-91-0428-TX-002 Date: June 30, 1992 GLP, Unpublished
Previous evaluation	In DAR (2000) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	mean weight [g]	mean length [cm]	Dura-tion	test method	water hard-ness*	pH	T [°C]	Crite-rion	Value	Unit	a/n	Ri
BRAVO 720	Lepomis macrochirus	0.39	3.0	96 h	flow-throug h	30-36	7.1-7.3	23	LC50	0.064	mg/l	a	2

* in mg/l as $CaCO_3$

Description

Bravo 720 is a liquid containing 53.6% chlorothalonil. Six test concentrations, 0.016 - 0.2 mg/l, plus control. Ten fish per vessel, in duplo. A 2 mg/l stock solution was prepared by vigorously mixing 100 mg formulation in 50 litres water for 2 hours. Actual concentrations chlorothalonil measured at 0 and 96 hours in all vessels by GC with electron capture detection (recovery 101%). Test according to EPA Guidelines.

Results

Mean measured concentrations were 45-78% of nominal at both t = 0 and 96 hours. Based on mean measured concentrations the 96-hours LC50 0.065 mg/l (95% confidence interval 0.050-0.094 mg/l) calculated using non-linear interpolation.

Remarks

Actual concentrations are <80% of nominal. A solution of Bravo720 at 2 mg/l solution has 1.07 mg chlorothalonil/l. The water solubility of chlorothalonil is 0.6 mg/l at 25°C, indicating that ca. 56% of the a.i. is in solution. The average mean measured concentration was 60% of nominal. The results in the heading table are recalculated with the trimmed Spearman-Karber method, based on Hamilton et al (1977/78): 95% confidence interval 0.057- 0.072 mg/l. The incipient LC50 is probably not reached. The result 96h LC50 0.064 mg/l is used for risk evaluation.

Report:	IIIA, 10.2.1/03 (numbering of Volume 2 original DAR). Shults, S. K. Brock, A. W., Laveglia, J., Machado, M. W., 1994. Acute toxicity to rainbow trout (*Oncorhynchus)* *mykiss*) under flow-through conditions with BRAVO[7] 720. Generated by: Ricerca, Inc. and Springborn Laboratories Inc. Submitted by: Zeneca Report No.: 5727-93-0120-TX-002 Date: July 7, 1994 GLP, Unpublished
Previous evaluation	In DAR (2000) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	mean weight [g]	mean length [cm]	Dura-tion	test method	water hard-ness*	pH	T [°C]	Crite-rion	Value	Unit	a/n	Ri
BRAVO 720	Oncorhynchus mykiss	0.56	3.9	96 h	flow-through	34-38	7.0-7.2	11-12	LC50	0.061	mg/l	a	2

* in mg/l as $CaCO_3$

Description

Bravo 720 is a liquid containing 54.5% chlorothalonil. Five test concentrations, 0.016 - 0.12 mg/l, plus control and a formulation-without-a.i.-control. Ten fish per vessel, in duplo. A 1.15 mg/l stock solution was prepared by vigorously mixing 57.5 mg formulation in 50 litres water for 2 hours. Actual concentrations chlorothalonil measured at 0 and 96 hours in all vessels by GC with electron capture detection (recovery 101%). Test according to EPA Guidelines.

Results

Mean measured concentrations were 68-77% of nominal at both t = 0 and 96 hours. Based on mean measured concentrations the 96-hours LC50 0.061 mg/l (95% confidence interval 0.049-0.089 mg/l) calculated using non-linear interpolation.

Remarks

Actual concentrations are <80% of nominal. A solution of Bravo720 at 1.15 mg/l solution has 0.63 mg chlorothalonil/l. The water solubility of chlorothalonil is 0.6 mg/l at 25°C, indicating that <96% (at 11-12°C) of the a.i. is in solution. The average mean measured concentration was 72% of nominal. The results in the heading table are recalculated with the trimmed Spearman-Karber method, based on Hamilton et al (1977/78): 95% confidence interval 0.056- 0.066 mg/l. The result 96h LC50 0.061 mg/l is used for risk evaluation.

Report:	IIIA, 10.2.1/01 (numbering in addendum 09 of 2001). S.E. Magor, . Shillabeer., 1999. Chlorothalonil: Acute toxicity to rainbow trout (*oncorhynchus mykiss*) of a 750 g/l WG formulation Generated by: Zeneca Research Submitted by: Zeneca Company file No.: BL6762/B date: December 1999
Previous evaluation	In addendum 09 to the DAR (2001) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Duration	Test type	pH	T [°C]	Criterion	Value	Unit	a/n	Ri
750 g/l WG	Oncorhynchus mykiss	96 h	Static	7.0-7.6	15	LC50	33	µg/l	a	2

Description

The toxicity of a WG formulation of chlorothalonil is tested at six test concentrations, 32, 56, 100, 180, 320, and 560 µg/l and a control. Test according to OECD Guidelines. Ten fish per concentration were kept under a light regime of 16h light- 8h dark and gently aerated. The mean length of the fish in the dilution water control at the end of the exposure period was 52 mm and the mean weight 2.1 g. Actual concentrations chlorothalonil were measured at 0, 48 and 96h; samples were extracted with hexane and extracts were analysed by GC-ECD, mean recoveries 92-98%.

Results

Actual concentrations chlorothalonil in the waterphase were 88-96% at t = 0h, after 96h they had decreased to <4% of nominal.

After 48h, 50% of the fish were dead at nominal concentrations of 100 µg/l and more. This mortality percentage remained stable during the rest of the study.

Remarks

Based on mean measured concentrations a 96-hours LC50 of 33.4 µg/l (95% confidence interval 24.8-45.1) can be calculated. The results in the heading table are used for risk evaluation.

Report: K-CP 10.2.1/01, Volz E., (2004). Acute toxicity of Azoxystrobin / Chlorothalonil SC (80/400) (A14111B) to rainbow trout (*Oncorhynchus mykiss*) in a 96-hour static test, Report Number 852016, RCC Ltd, Environmental Chemistry & Pharmanalytics, CH-4452 Itingen / Switzerland. (Syngenta File No. ICI5504/2322)	
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. Based on mean measured concentrations, the 96-hour LC_{50} of A14111B to

	rainbow trout was 0.061 mg product/L, corresponding to 0.021 mg chlorothalonil/L.

Guidelines: OECD No. 203; EU Commission Directive 9269/EEC, C.1

GLP: Yes

Executive Summary

Juvenile rainbow trout (*Oncorhynchus mykiss)* were exposed to nominal concentrations of 0.046, 0.10, 0.22, 0.46 and 1.0mg A14111B/L in a static test design for 96 hours.

Based on mean measured concentrations, the 96-hour LC_{50} of A14111B to rainbow trout was 0.061 mg product/L, corresponding to 0.021 mg chlorothalonil/L.

Materials

Test Material:	A14111B
Description:	Cream opaque liquid
Lot/Batch #:	J7518/024
Purity:	80 g/L azoxystrobin (6.6% w/w) and 419 g/L chlorothalonil (34.6% w/w)
Stability of test compound:	Assumed stable pending re-analysis in September 2005
Density:	1.21 g/mL
Test concentrations:	Dilution water control and nominal formulation concentrations of 0.046, 0.10, 0.22, 0.46 and 1.0mg A14111B/L
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes (based on measurement of chlorothalonil)
Test animals	
Species:	Rainbow trout *Oncorhynchus mykiss*
Source:	P. Hohler, trout breeding station, Zeiningen, Switzerland
Acclimatisation period:	One week
Treatment for disease:	None
Weight and length of fish:	Weight: range 1.2 $\pm$ 0.1 g Length: range 5.0 $\pm$ 0.2 cm
Feeding:	None during test
Environmental conditions	
Test temperature:	14°C throughout the test

pH range:	7.6 to 7.8
Dissolved oxygen:	9.2 – 9.9 mg/L
Total hardness of dilution water:	2.5 mmol/L (=250 mg/L) as $CaCO_3$
Lighting:	16 hours fluorescent light (50-500 Lux) and 8 hours dark with 30 minute dawn and dusk transition periods
Length of test:	96 hours

Study Design and Methods

Experimental dates: 23rd January to 11th February 2004

Juvenile rainbow trout (*Oncorhynchus mykiss)* were exposed to nominal concentrations of 0.046, 0.10, 0.22, 0.46 and 1.0mg A14111B/L in a static test design for 96 hours. There was one replicate containing 7 fish in an untreated control and at each test concentration. The test water was a reconstituted water. All glass aquaria were filled with 15 litres of test medium. The test media and the control vessels were slightly aerated during the test period.

Concentrations of chlorothalonil were analysed at 0 hours (to ensure correct preparation of nominal concentrations) and at 96 hours (or earlier in case all fish died). The behaviour and survival of the fish were assessed at 3, 24, 48, 72 and 96 hours after initiation of the test. Temperature, dissolved oxygen, and pH were measured at 24-hour intervals. The test was conducted under static conditions.

Results and Discussion

The measured concentrations of chlorothalonil at the start of the test ranged from 81 to 108% of the nominal values and at the end of the test ranged from 6.1 to 64%. Results were based on nominal concentrations, which is not acceptable as concentrations were not maintained at >80% of nominal. RMS calculated the LC_{50} value based on geometric mean measured chlorothalonil concentrations using probit analysis; the resulting LC_{50} is similar to the geometric mean of the concentrations with 0 and 100% mortality, as there were no concentrations with intermediate mortality. Confidence intervals could not be obtained.

Table 10.2.1-1: Concentrations based on the quantification of chlorothalonil technical

Nominal concentration (mg A14111B/L)	**Nominal mg Chlorothalonil Technical/L**	**Measured mg chlorothalonil technical/L**				
		At t=0 (mean of 2 measurements)	% of nominal	At test end (mean of 2 measurements)	% of nominal	Geometric mean
Control	0	-	-	-	-	-
0.046	0.016	0.017	107	< LOQ	22	0.0078
0.10	0.035	0.034	98	0.0021	6.1	0.0084
0.22	0.076	0.067	88	0.039	51	0.051
0.46	0.16	0.15	93	0.10	62	0.12
1.0	0.35	0.34	100	0.19	55	0.26

The effects of A14111B upon mortality of rainbow trout and the LC_{50} values are shown in Table 10.2.1-2.

Table 10.2.1-2: Effects of A14111B upon mortality of rainbow trout and LC_{50} values

Nominal concentration (mg A14111B/L)	**Mean measured concentration mg chlorothalonil/L**	**Cumulative mortality (out of 7)**				
		3h	24h	48h	72h	96h
Control	control	0	0	0	0	0
0.046	0.0078	0	0	0	0	0
0.10	0.0084	0	0	0	0	0
0.22	0.051	0	5	7	7	7
0.46	0.12	0	7	7	7	7
1.0	0.26	0	7	7	7	7
LC_{50} values (mg A14111B/L)						0.061
95% Confidence Limits						
LC_{50} value (mg chlorothalonil/L)						0.021

nc – not calculable

Valitity of the test:

- Mortality in the controls was < 10% (i.e. 0%).
- Constant conditions were maintained throughout the test
- The dissolved oxygen concentration was > 60% of the air saturation value throughout the test (i.e. > 9.2 mg O_2/L)

Conclusion

Based on mean measured concentrations, the 96-hour LC_{50} of A14111B to rainbow trout was 0.021 mg chlorothalonil/L, corresponding to 0.061 mg product/L (based on analysed content of chlorothalonil in product).

Report:	IIIA, 10.2.1/01 (numbering of Volume 2 original DAR). Wütrich, V., 1989. 24-Hour acute toxicity of Daconil 2787[7] Extra to *Daphnia magna* (OECD-immobilization test). Generated by: RCC Umweltchemie AG Submitted by: Zeneca Report No.: 258074 Date: December 15, 1989 GLP, Unpublished
Previous evaluation	In DAR (2000) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Age	Duration	test type	water hardness*	pH	T [°C]	Criterion	Value	Unit	a/n	Ri
Daconil 2787 Extra	Daphnia magna	<24 h	48 h	static	238	8.2-8.5	22	EC50	0.86	mg/l	n	1

***** in mg/l as $CaCO_3$

Description

Daconil 2787 Extra is a suspension concentrate containing 40.4% chlorothalonil. Ten test concentrations, 0.063 - 10 mg/l, plus control. Ten daphnids per vessel, two vessels per concentration. Actual concentrations were not measured. Test according to OECD202.

Results

48-hours EC50 0.88 mg/l; (95% confidence interval 0.71-1428 mg/l) calculated with logit method.

Remarks

The results in the heading table are calculated using data from author, with the trimmed Spearman-Karber method, based on Hamilton et al (1977/78): 95% confidence interval 0.77-0.96 mg/l. The result

48h EC50 0.86 mg/l is not used for risk evaluation, because it is based on nominal concentrations, which is not acceptable as concentrations were not maintained at >80% of nominal..

Report:	IIIA, 10.2.1/01 (numbering of Volume 2 original DAR). Gelin, M. D., Laveglia, J., Putt, A. E.., 1992. BRAVO 720 - Acute toxicity to daphnids (*Daphnia magna*). Generated by: Ricerca, Inc. and Springborn Laboratories Inc. Submitted by: Zeneca Report No.: 5087-91-0427-TX-002 Date: June 30, 1992 GLP, Unpublished
Previous evaluation	In DAR (2000) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Age	Duration	test type	water hardness*	pH	T [°C]	Criterion	Value	Unit	a/n	Ri
BRAVO 720	Daphnia magna	<24 h	48 h	static	170	8.2-8.3	19-21	EC50	0.179	mg/l	a	1

***** in mg/l as $CaCO_3$

Description

BRAVO 720 is a liquid containing 54% chlorothalonil. Ten test concentrations, 0.065 - 0.5 mg/l, plus control. Ten daphnids per vessel, two vessels per concentration. Actual concentrations were measured at 0 and 48 hours by GC-EC (recovery 101%). Test according to EPA Guidelines.

Results

Mean measured concentrations were 83-89% of nominal, except the 65 µg/l level: 77%. 48-hours EC50 0.180 mg/l (95% confidence interval 0.16-0.20 mg/l) calculated with probit analysis.

Remarks

The results in the heading table are calculated using data from author, with the trimmed Spearman-Karber method, based on Hamilton et al (1977/78): 95% confidence interval 0.16-0.20 mg/l. The incipient LC50 is not reached. The result 48h EC50 0.179 mg/l is used for risk evaluation.

Report:	IIIA, 10.2.1/01 (numbering in addendum 09 of 2001). S.E. Magor, N. Shillabeer, 1999. Chlorothalonil: Acute Toxicity to *Daphnia magna* of a 750 g/l WG formulation. Generated by: Zeneca Research Submitted by: Zeneca Company file No.: BL6763/B date: December 1999
Previous evaluation	In addendum 09 to the DAR (2001) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Duration	test type	pH	T [°C]	Criterion	Value	Unit	A/n	Ri
750 g/l WG	Daphnia magna	48 h	Static	7.9-8.1	20	EC50	112	µg/l	a	1

Description

The toxicity of a WG formulation of chlorothalonil to *D.magna* (<24h old) is tested at seven test concentrations, 20, 40, 80, 160, 320, 640 and 1300 µg/l and a control. Test according to OECD Guidelines. Actual concentrations chlorothalonil were measured at the start and at the end of the test; samples were extracted with hexane and extracts were analysed by GC-ECD, mean recoveries 96-98%.

Results

Actual concentrations chlorothalonil in the waterphase were 88-93% at t = 0h, after 96h they had decreased to 31-93% of nominal.

Remarks

The 48-hours EC50 of 112 µg/l (95% confidence interval 89-141) based on mean measured concentrations in the heading table is recalculated using the Spearman-Kärber method. The results in the heading table are used for risk evaluation.

Report: K-CP 10.2.1/02, Volz E., (2004a). Acute toxicity of Azoxystrobin / Chlorothalonil SC (80/400) (A14111B) to *Daphnia magna* in a 48-hour immobilisation test, Report Number 852019, RCC Ltd, Environmental Chemistry & Pharmanalytics, CH-4452 Itingen / Switzerland. (Syngenta File No. ICI5504/2240)	
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. Based on geometric mean measured chlorothalonil concentrations, the 48-hour EC_{50} of A14111B to *Daphnia magna* was 0.12 mg chlorothalonil/L , corresponding to 0.35 mg product/L (based on analysed content of chlorothalonil in product). The EC_{10} and EC_{20} were 0.10 and 0.11 mg

	chlorothalonil/L, corresponding to 0.29 and 0.32 mg product/L.

Guidelines: OECD No. 202

GLP: Yes

Executive Summary

First instar *Daphnia magna* were exposed to nominal concentrations of 0.046, 0.10, 0.22, 0.46, 1.0 and 2.2 mg A14111B/L in a static test design for 48 hours. There were four replicates, each containing 5 daphnids, in an untreated control and at each test concentration.

Based on geometric mean measured chlorothalonil concentrations, the 48-hour EC_{50} of A14111B to *Daphnia magna* was 0.12 mg chlorothalonil/L , corresponding to 0.35 mg product/L (based on analysed content of chlorothalonil in product). The EC_{10} and EC_{20} were 0.10 and 0.11 mg chlorothalonil/L, corresponding to 0.29 and 0.32 mg product/L.

Materials

Test Material:	A14111B
Description:	Cream opaque liquid
Lot/Batch #:	J7518/024
Purity:	80 g/L azoxystrobin (6.6%) and 419 g/L chlorothalonil (34.6%)
Stability of test compound:	Assumed stable pending re-analysis in September 2005
Density:	1.21 g/mL
Test concentrations:	Dilution water control and nominal formulation concentrations of 0.046, 0.10, 0.22, 0.46 and 1.0mg A14111B/L
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes (based on measurement of chlorothalonil)
Test animals	
Species:	*Daphnia magna*
Source:	In house source
Treatment for disease:	None
Feeding:	None during test
Environmental conditions	
Test temperature:	20°C throughout the test
PH range:	7.9 to 8.1
Dissolved oxygen:	8.9 mg/L
Total hardness of dilution water:	2.5 mmol/L (=250 mg/L) as $CaCO_3$
Lighting:	16 hours fluorescent light (590-710 Lux) and 8 hours dark with 30 minute dawn and dusk transition periods
Length of test:	48 hours

Study Design and Methods

Experimental dates: 26th January to 12th February 2004

First instar *Daphnia magna* were exposed to nominal concentrations of 0.046, 0.10, 0.22, 0.46, 1.0 and 2.2 mg A14111B/L in a static test design for 48 hours. There were four replicates, each containing 5 daphnids, in an untreated control and at each test concentration.

Concentrations of chlorothalonil were analysed at 0 hours (to ensure correct preparation of nominal concentrations) and at 96 hours. *Daphnia* immobility was assessed 24 and 48 hours after initiation of the test. Dissolved oxygen, pH and temperature were measured at 0 and 48 hours. The test was conducted under static conditions.

Results and Discussion

The measured concentrations of chlorothalonil at the start of the test ranged from 64 to 99% of the nominal values and at the end of the test ranged from 39 to 118%. Results were based on nominal concentrations which is not acceptable as concentrations were not maintained at >80% of nominal. RMS calculated the EC_{10}, EC_{20} and EC_{50} values based on mean measured chlorothalonil concentrations using Toxrat v3.0.0. Confidence intervals could not be obtained due to mathematical reasons.

Table 10.2.1-3: Concentrations based on the quantification of chlorothalonil technical

Nominal concentration (mg A14111B/L)	**Nominal mg Chlorothalonil Technical/L**	**Measured mg chlorothalonil technical/L**				
		At t=0 (mean of 2 measurements)	% of nominal	At test end (mean of 2 measurements)	% of nominal	Geometric mean
Control	0	-	-	-	-	-
0.046	0.016	0.012		-*	-	-
0.10	0.035	0.027		-*	-	-
0.22	0.076	0.055	72	0.055	72	0.055
0.46	0.16	0.15	94	0.14	90	0.14
1.0	0.35	0.34	97	0.34	100	0.34
2.2	0.76	0.75	99	0.35	47	0.51

*The samples of the nominal test concentrations of 0.046 and 0.1 mg product/L were not analysed after 48 hours since the concentrations were below the 48h NOEC determined in this test.

The effects of A14111B upon immobilisation of *Daphnia magna* and the EC_{50} values are presented in Table 10.2.1-4.

Table 10.2.1-4: Effects of A14111B upon immobilisation of *Daphnia magna* and EC_{50} values

Nominal concentration (mg A14111B/L)	**Immobility (%)**	
	24h	48h
Control	0	0
0.046	0	5
0.10	0	0
0.22	0	0

Nominal concentration (mg A14111B/L)	Immobility (%)	
0.46	60	85
1.0	85	100
2.2	95	100

Valitity criteria of the test were met:

- Immobility in the controls was < 10% (i.e. 0%).
- The dissolved oxygen concentration was > 60% of the air saturation value throughout the test (i.e. 8.9 mg O_2/L)

Conclusion

Based on geometric mean measured chlorothalonil concentrations, the 48-hour EC_{50} of A14111B to *Daphnia magna* was 0.12 mg chlorothalonil/L , corresponding to 0.35 mg product/L (based on analysed content of chlorothalonil in product). The EC_{10} and EC_{20} were 0.10 and 0.11 mg chlorothalonil/L, corresponding to 0.29 and 0.32 mg product/L.

Report:	IIIA, 10.2.1/05 (numbering of Volume 2 original DAR). Wütrich, V., 1990. Acute toxicity of Daconil 2787[7] Extra to *Scenedesmus subspicatus* (OECD-algae growth inhibition test). Generated by: RCC Umweltchemie AG Submitted by: Zeneca Report No.: 258085 Date: March 2, 1990 GLP, Unpublished
Previous evaluation	In DAR (2000) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Duration	test type	pH	T [°C]	Criterion	Value	Unit	a/n	Ri
Daconil 2787 Extra	Scenedesmus subspicatus	72 h	static	7.7	24	NOEC EbC50	0.016 0.53	mg/l mg/l	a n	2 2

Description

Daconil 2787 Extra is a suspension concentrate containing 40.4% chlorothalonil. Five test concentrations, 0.04, 0.16, 0.63, 2.5, and 10 mg/l, plus control. Actual concentrations were measured by GC (recovery 90%) at t = 0 and 96 hours in the 0.04, 0.63, and 10 mg/l vessels, as well as in a 10 mg/l vessel without algae. Test according to OECD201 Guidelines.

Results

At initiation the actual concentrations were 59-80% of nominal; at termination 0 - 64% of nominal. The 10 mgl vessel without algae contained 78 and 74% of nominal at t = 0 and 96 hours. The pH (initially 7.7) had dropped to 3.7 - 4.1 at 0 - 0.63 mg/l. At 2.5 and 10 mg/l the pH was 7.9 after 96 hours. The results are calculated by author using nominal concentrations (logit analysis): 72-hours E_bC50 0.521 mg/l; 96-hours E_bC50 0.535 mg/l. With Dunnett's test the NOEC is 0.04 mg/l (9% inhibition).

Remarks

pH change is 3-4 log-units and RCC (test lab) suggests that this is very probable caused by the extremely high algal growth. Furthermore, the pH deviaton during the test should normally be within one unit, but this is only a recommendation, not a validity criterion.

Actual concentrations <80% of nominal, but the performance of the test probably cannot be improved on this point. The E_bC50 in the heading table is recalculated using nominal data from author with a log-logistic regression: 72-hours EbC50 0.53 mg/l (0.38 - 0.74 mg/l), 96-hours EbC50 0.66 mg/l (0.32-1.33 mg/l). NOEC based on mean measured concentrations is 0.016 mg/l. The results in the heading table are not used for risk evaluations.

Report:	IIIA, 10.2.1/01 (numbering in addendum 09 of 2001). Smyth, D.V., S.E. Magor, . Shillabeer., 1999. Chlorothalonil: Acute toxicity to the green alga *Selenastrum capricornutum* of a 750 g/l WG formulation Generated by: Zeneca Research Submitted by: Zeneca Company file No.: BL6761/B date: December 1999
Previous evaluation	In addendum 09 to the DAR (2001) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Duration	test type	PH	T [°C]	Criterion	Value	Unit	a/n	Ri
750 g/l WG	Selenastrum capricornutum	72 h	Static	7.3-9.4	24	EbC50 ErC50 NOEbC	110 330 19	µg/l µg/l µg/l	n	1

Description

A WG formulation of chlorothalonil (content 75%) is tested at eight test concentrations, 3.9, 8.6, 19, 41, 91, 200, 450, and 1000 µg/l and a control. Test according to OECD Guidelines. A three day old culture was used as inoculum. Test solutions were incubated in triplicate under continuous light (8040 lux) with orbital shaking at 160 rpm. Actual concentrations chlorothalonil were measured for all test solutions at the start and for the blank solution at the end of the test; samples were extracted with hexane and extracts were analysed by GC-ECD, mean recoveries 94-98%.

Results

Actual concentrations chlorothalonil were 88-97% of nominal during the study.

Remarks

At a concentration of 91 µg/l the mean area under the growth curve was reported to be significantly different from the control. Recalculation of the data shows a significant difference for 41 µg/l as well.The recalculated NOEbC is 19 µg/l. The results in the heading table are used for risk evaluation.

Report: K-CP 10.2.1/03, Volz E., (2004b). Toxicity of Azoxystrobin / Chlorothalonil SC (80/400) (A14111B) to *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a 96-hour algal growth inhibition test, Report Number 852022, RCC Ltd, Environmental Chemistry & Pharmanalytics, CH-4452 Itingen / Switzerland. (Syngenta File No. ICI5504/2239)	
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. Reported endpoints were based on nominal and measured initial concentrations, which is not acceptable as concentrations were not maintained stable during the exposure period. RMS re-calculated EC_{50}, EC_{20}, and EC_{10} values for growth rate, biomass and yield based on geometric mean measured concentrations. As effect concentrations of the 72 hour period were lower than for the 96 hour period, only the results of the 72 hour period are used for further risk assessments. The 72-hour ErC_{50} was 0.20 mg chlorothalonil/L (95% CI 0.17-0.23 mg chlorothalonil/L), corresponding to 0.58 mg product/L . ErC_{10} and ErC_{20} were 0.025 mg chlorothalonil/L (95% CI 0.018-0.033 mg chlorothalonil/L) and 0.051 mg chlorothalonil/L (95% CI 0.040-0.062 mg chlorothalonil/L), respectively, corresponding to 0.072 and 0.15 mg product/L, respectively. The EbC_{50}, EbC_{20} and EbC_{10} were calculated to be 0.037 mg chlorothalonil/L (95% CI 0.030-0.044 mg chlorothalonil/L), 0.0090 mg chlorothalonil/L (95% CI 0.0061-0.012 mg chlorothalonil/L) and 0.0044 mg chlorothalonil/L (95% CI 0.0026-0.0064 mg chlorothalonil/L), respectively, corresponding to 0.11, 0.026 and 0.013 mg product/L, respectively. EyC_{10} 0.0037 mg chlorothalonil/L (95% CI 0.0019-0.0057 mg chlorothalonil/L), EyC_{20} 0.0077 mg chlorothalonil/L (95% CI 0.0048-0.011 mg chlorothalonil/L) and EyC_{50} 0.033 mg chlorothalonil/L (95% CI 0.026-0.040 mg chlorothalonil/L), corresponding to respectively 0.011, 0.022 and 0.095 mg product/L.

Guidelines: OECD 201 (1984) and EC L 383 A, Part C.3

GLP: Yes

Executive Summary

Pseudokirchneriella subcapitata, inoculated at 1.0×10^4 cells/mL, was cultured in concentrations of A14111B in sterile culture medium at 24°C for 96 hours under static conditions. The nominal

concentrations employed were 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg formulation/L. Based on geometric mean measured concentrations of chlorothalonil, the effect concentratiosn were as follows:

Parameter	**After 72 hours**			**After 96 hours**		
	Biomass, b (mg/L)	**Yield, y (mg/L)**	**Growth rate, r (mg/L)**	**Biomass b (mg/L)**	**Yield, y (mg/L)**	**Growth rate, r (mg/L)**
EC_{50}	0.037	0.033	0.20	0.053	0.069	0.42
95%-confidence limits	0.030 – 0.044	0.026-0.040	0.17 – 0.23	0.044 – 0.063	0.058-0.082	0.37 –0.47
EC_{10}	0.0044	0.0037	0.025	0.0088	0.015	0.039
95% CI	0.0026-0.0064	0.0019-0.0057	0.018-0.033	0.0055-0.012	0.0092-0.020	0.030-0.049
EC_{20}	0.0090	0.0077	0.051	0.016	0.025	0.088
95% CI	0.0061-0.012	0.0048-0.011	0.040-0.062	0.012-0.021	0.018-0.031	0.072-0.10
NOEC	< 0.011	< 0.011	< 0.011	< 0.011	0.011	0.034

Materials

Test Material:	A14111B
Description:	Cream opaque liquid
Lot/Batch #:	J7518/024
Purity:	80 g/L azoxystrobin (6.6%) and 419 g/L chlorothalonil (34.6% w/w)
Stability of test compound:	Assumed stable pending re-analysis in September 2005
Density:	1.21 g/mL
Test concentrations:	Dilution water control and nominal formulation concentrations of 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg formulation/L
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes (based on measurement of chlorothalonil)
Test organism	
Species:	*Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), Strain No. 61.81 SAG
Source:	SAG, Institute for Plant Physiology, University of Göttingen, Germany
Environmental conditions	
Test temperature:	23-24 °C
PH range:	7.9 to 8.0 at the start of the test and from 8.0 to 9.0 at the end of the test
Lighting:	8000 to 9100 lux.
Length of test:	96 hours

Study Design and Methods

Experimental dates: 20th February to 11th March, 2004.

Pseudokirchneriella subcapitata, inoculated at 1.0×10^4 cells/mL, was cultured in concentrations of A14111B in sterile culture medium at 24°C for 96 hours. The nominal concentrations employed were 0.032, 0.10, 0.32, 1.0, 3.2 and 10 mg formulation/L. Six replicate cultures of the culture medium control and triplicate cultures of each concentration of formulation were prepared.

Concentrations of chlorothalonil were analysed at 0 hours (to ensure correct preparation of nominal concentrations) and at 96 hours. Algal cell numbers were determined after 24, 48, 72 and 96 hours. The pH was measured at the start and end of the study, temperature was measured daily and light intensity once during the study. The test was conducted under static conditions.

Results and Discussion

The measured concentrations of azoxystrobin at the start of the test ranged from 83 to 104% of the nominal values and at the end of the test ranged from 68 to 101 %. Results were expressed in nominal concentrations, which is not acceptable as concentrations were not maintained at >80% of nominal. RMS calculated all effect concentrations for biomass, yield and growth rate based on geometric mean measured chlorothalonil concentrations using Toxrat v3.0.0.

The results are shown in Table 10.2.1-5 to 10.2.1-8.

Table 10.2.1-5: Concentrations based on the quantification of chlorothalonil technical

Nominal concentration (mg A14111B/L)	Nominal mg Chlorothalonil Technical/L	Measured mg chlorothalonil technical/L				
		At t=0 (mean of 2 measurements)	% of nominal	At test end (mean of 2 measurements)	% of nominal	Geometric mean
Control	0	-	-	-	-	-
0.032	0.011	0.0115	109	0.0113	100	0.011
0.10	0.035	0.0362	103	0.0321	94	0.034
0.32	0.11	0.108	100	0.0838	76	0.095
1.0	0.35	0.312	88	0.237	69	0.27
3.2	1.1	0.956	86	0.821	75	0.89
10	3.5	2.865	83	2.99	86	2.9

Table 10.2.1-6: A14111B - Areas under the algal growth curves (AUC) and percentage inhibition of AUC (I_{AUC}) during the test period

Nominal concentration (mg A14111B/L)	Mean areas under the growth curves (AUC) and % inhibition of biomass							
					0-72 h		0-96 h	
					AUC	I_{AUC} (%)	AUC	I_{AUC} (%)
Control					1902	0.0	6844	0.0
0.032					1310	31.1*	5411	20.9*
0.10					1189	37.5*	4710	31.2*
0.32					470	75.3*	2336	65.9*
1.0					152	92.0*	522	92.4*
3.2					39	97.9*	81	98.8*
10					66	96.6*	128	98.1*

* Significant difference (P=0.05) from the culture medium control (Williams Multiple Sequential t-test, one-sided smaller)

Table 10.2.1-7: A14111B - Algal growth rates (r) and percentage inhibition of r (Ir) during the test period

Nominal concentration (mg A14111B/L)	Growth rate r and % inhibition of r							
					0-72 h		0-96 h	
					r (1/day)	I_r (%)	r (1/day)	I_r (%)
Control					1.55	0.0	1.43	0.0
0.032					1.40	9.7*	1.40	2.2
0.10					1.37	11.7*	1.35	5.3
0.32					1.08	30.3*	1.21	15.0*
1.0					0.65	58.0*	0.79	44.7*
3.2					0.16	89.9*	0.33	76.6*
10					0.23	85.0*	0.41	71.3*

* Significant difference (P=0.05) from the culture medium control (Williams Multiple Sequential t-test, one-sided smaller)

The effect concentrations for *Pseudokirchneriella subcapitata* exposed to A14111B (based on geometric mean measured concentration of chlorothalonil) are presented in Table 10.2.1-8.

Table 10.2.1-8: EC values for *Pseudokirchneriella subcapitata* exposed to A14111B

Parameter	After 72 hours			After 96 hours		
	Biomass, b (mg/L)	Yield, y (mg/L)	Growth rate, r (mg/L)	Biomass b (mg/L)	Yield, y (mg/L)	Growth rate, r (mg/L)
EC_{50}	0.037	0.033	0.20	0.053	0.069	0.42
95%-confidence limits	0.030 – 0.044	0.026-0.040	0.17 – 0.23	0.044 – 0.063	0.058-0.082	0.37 –0.47
EC_{10}	0.0044	0.0037	0.025	0.0088	0.015	0.039
95% CI	0.0026-0.0064	0.0019-0.0057	0.018-0.033	0.0055-0.012	0.0092-0.020	0.030-0.049
EC_{20}	0.0090	0.0077	0.051	0.016	0.025	0.088
95% CI	0.0061-0.012	0.0048-0.011	0.040-0.062	0.012-0.021	0.018-0.031	0.072-0.10
NOEC	< 0.011	< 0.011	< 0.0114	< 0.011	0.011	0.034

n.d. not determined

Validity criteria of OECD 201 (1984) were met:

- the cell concentration in the control cultures were increased by a factor of > 16 within 3 days (i.e. 108)

The additional validity criteria of OECD 201 (2006) were also met for the 72 hour period.

- The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 16.7%, which is < the criterion of 35%
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was < 7% (i.e. 4.5).

As the effect concentrations of the 72 hour period are lower than for the 96 hour period, only the results of the 72 hour period are used for further risk assessments.

Conclusion

Reported endpoints were based on nominal and measured initial concentrations, which is not acceptable as concentrations were not maintained stable during the exposure period.

RMS re-calculated EC_{50}, EC_{20}, and EC_{10} values for growth rate, biomass and yield based on geometric mean measured concentrations. As effect concentrations of the 72 hour period were lower than for the 96 hour period, only the results of the 72 hour period are used for further risk assessments.

The 72-hour ErC_{50} was 0.20 mg chlorothalonil/L (95% CI 0.17-0.23 mg chlorothalonil/L), corresponding to 0.58 mg product/L . ErC_{10} and ErC_{20} were 0.025 mg chlorothalonil/L (95% CI 0.018-0.033 mg chlorothalonil/L) and 0.051 mg chlorothalonil/L (95% CI 0.040-0.062 mg chlorothalonil/L), respectively, corresponding to 0.072 and 0.15 mg product/L, respectively.

The EbC_{50}, EbC_{20} and EbC_{10} were calculated to be 0.037 mg chlorothalonil/L (95% CI 0.030-0.044 mg chlorothalonil/L), 0.0090 mg chlorothalonil/L (95% CI 0.0061-0.012 mg chlorothalonil/L) and 0.0044 mg chlorothalonil/L (95% CI 0.0026-0.0064 mg chlorothalonil/L), respectively, corresponding to 0.11, 0.026 and 0.013 mg product/L, respectively.

EyC_{10} 0.0037 mg chlorothalonil/L (95% CI 0.0019-0.0057 mg chlorothalonil/L), EyC_{20} 0.0077 mg chlorothalonil/L (95% CI 0.0048-0.011 mg chlorothalonil/L) and EyC_{50} 0.033 mg chlorothalonil/L (95% CI 0.026-0.040 mg chlorothalonil/L), corresponding to respectively 0.011, 0.022 and 0.095 mg product/L.

B.9.3.2 Additional long-term and chronic toxicity to fish, aquatic invertebrates and sediment dwelling organisms

Report:	IIIA, 10.2.1/02 (numbering of Volume 2 original DAR). Voigt, I.A., 1989. Toxizität von Daconil 2787 führ Regenbogenforellen *Salmo gairdnerii* bei verlängerter Exposition (21 Tage). Generated by: Ökolimna Submitted by: Zeneca Report No.: 09/89/118 Date: December 21, 1989 GLP, Unpublished
Previous evaluation	In DAR (2000) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	mean weight [g]	mean length [cm]	Dura-tion	test method	water hard-ness*	pH	T [°C]	Crite-rion	Value	Unit	a/n	Ri
Daconil 2787 Extra	Oncorhynchus mykiss	1.5	5.6	21 d	semi-static	233	7.7-8.2	14-16	NOEC	0.00087	mg/l	a	2

* in mg/l as $CaCO_3$

Description

Daconil 2787 Extra is a suspension concentrate containing 40.4% chlorothalonil. Six test concentrations, 0.0023, 0.0049, 0.0106, 0.0227, 0.049, and 0.1056 mg/l, plus control. Ten fish per vessel. Renewal of medium every 3-4 days. Actual concentrations chlorothalonil were measured 5 times in 21 days by HPLC (recovery 87-100%), except for the 0.1056 mg/l. Test according to OECD 204.

Results

Actual concentrations were 0 - 80% of nominal. Mean measured concentrations were 0.87, 1.98, 7.5, 12.8, and 19.6 µg/l.

No mortalities at 0.87 and 7.5 µg/l. At 1.98 µg/l 2 fish died. At 12.8, 19.6, and 105.6 µg/l 1, 3 and 10 fish died. Surviving fish at 1.98 µg/l onwards showed symptoms e.g. increased respiration, surfacing, nervous behaviour, reduced feeding. No influence on body weight and body length at any concentration. NOEC (mortality) 0.00087 mg/l (nominal 2.3 µg/l).

Remarks

Actual concentrations chlorothalonil <80%. The result NOEC 0.00087 mg/l (mean measured concentration) is used for risk evaluation.

Report:	IIIA, 10.2.1/04 (numbering of Volume 2 original DAR). Coenen, T.M.M., 1989. *Daphnia magna* reproduction test with Daconil Extra. Generated by: RCC and BCO Submitted by: Zeneca Report No.: 025751 Date: December 15, 1989 GLP, Unpublished
Previous evaluation	In DAR (2000) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Duration	test type	water hardness *	pH	T [°C]	Criterion	Value	Unit	a/n	Ri
Daconil 2787 Extra	Daphnia magna	22 d	semi-static	200	7.9-8.5	18-20	NOEC	<0.0023	mg/l	actual	1

*in mg/l as $CaCO_3$

Description

Daconil 2787 Extra is a suspension concentrate containing 40.4% chlorothalonil. Six test concentrations, 0.0019 - 0.56 mg/l, plus control. Renewal every two days. Ten daphnids per vessel, four vessels per concentration. Actual concentrations were measured by GC (recovery 93 - 101%) in fresh solutions (0.0018, 0.018, 0.18, and 0.56 mg/l) at 3 and 19 days, and in old solutions at 5 and 22 days. Test according to OECD202 Guideline.

Results

Actual concentrations after renewal were 117 - 211% of nominal. Actual concentrations before renewal were 19 - 111% of nominal. Mean measured concentrations were 0.00228, 0.0164, 0.2007, 0.588 mg/l.

Based on mortality of the parent generation (first week 2.5 - 100%; reproduction phase 10-40%) the NOEC is <0.0018 mg/l (nominal). Based on reproduction parameters the NOEC is 0.18 mg/l.

Remarks

The number of offspring in the 0.056 en 0.18 mg/l vessels is lower compared to control at 10 and 17 days, but at 22 days the difference was less obvious. NOEC reproduction 0.018 mg/l nominal, 0.016 mg/l mean measured.

The result 22-days NOEC <0.0023 mg/l (mean measured concentration) is used for risk evaluation.

B.9.4 Risk assessment for aquatic organisms

In Volume 1, section 2.9.2.1 an overview of the available endpoints for aquatic organisms is given and the relevant endpoints for the risk assessment are determined. From the acute data for fish, aquatic invertebrates and primary producers for the formulation A14111B, it appears that the formulation is not

more toxic to these taxonomic groups than the active substance. Hence, the risk assessment is based on the data from the active substance.

The risk assessment for aquatic organisms has been conducted in line with the EFSA Aquatic Guidance Document (EFSA, 2013).

Metabolites

The environmental fate assessment has identified SDS-3701 (R182281), SDS-46851 (R611965), R417888, R613841, R613842 and R613801 as potentially relevant metabolites in surface water (See B.8).

Acute and chronic risk assessment was carried out using the initial PEC_{SW} calculated with FOCUS Surface Water, Step 2, and, where required, FOCUS Step 3 and 4, and the lowest available toxicity endpoints for fish, invertebrates and primary producers. The resulting TER-values are presented for the different crops for chlorothalonil and the metabolites. TERs that do not meet the trigger of 10 for primary producers and the trigger of 100 for invertebrates and fish for the acute risk and the trigger of 10 for the chronic risk for invertebrates and fish are presented in bold. Higher tier risk assessments are performed where necessary. For PECsw estimations the reader is refered to section B.8.5.

B.9.4.1 Exposure

Aquatic organisms may be exposed to chlorothalonil and its major metabolites through spray drift, run-off and drainage from the application site into adjacent water bodies. Exposure of aquatic organisms from these routes was estimated by calculating Predicted Environmental Concentrations in surface water (PEC_{SW}) (see section B.8.5 for details of calculations).

B.9.4.2 Acute risk

First tier risk assessment based on FOCUS Step 2 exposure values

The acute Toxicity-Exposure Ratios (TERs) for the use in winter- and spring cereals and tomatoes are calculated with FOCUS Step 2 values. The TERs are presented in the table below.

Table B.9.4-01 Acute Toxicity-Exposure Ratios for aquatic organisms based on max FOCUS Step 2 PEC values

Species	**L(E)C_{50}** or **NOEC [µg as/L]**	**Cereals** **Max FOCUS Step 2**	**Tomatoes** **Max FOCUS Step 2**
		18.3 µg as/L 0.237 mg/kg sed	9.20 µg as/L 0.098 mg/kg sed
Acute			

Fish	16	**0.87**	**1.74**
Amphibians	8.2	**0.44**	**0.89**
Invertebrates	5	**0.27**	**0.54**
Algae	13	**0.71**	**1.41**
Aquatic plants	134	**7.32**	14.6

Based on FOCUS Step 2 PECsw values, the acute triggers (100) for aquatic vertebrates (fish and amphibians) and aquatic invertebrates are not met for all uses. Also the trigger of 10 for algae and aquatic plants is not met for all uses. Further refinement of the risk assessment for all taxonomic groups is necessary.

Refined acute risk to aquatic vertebrates (fish and amphibians) for chlorothalonil

The acute risk to aquatic vertebrates requires refinement. Table B.9.4-02 contains the acute toxicity data for 10 fish species. Furthermore, data from two amphibian species is available.

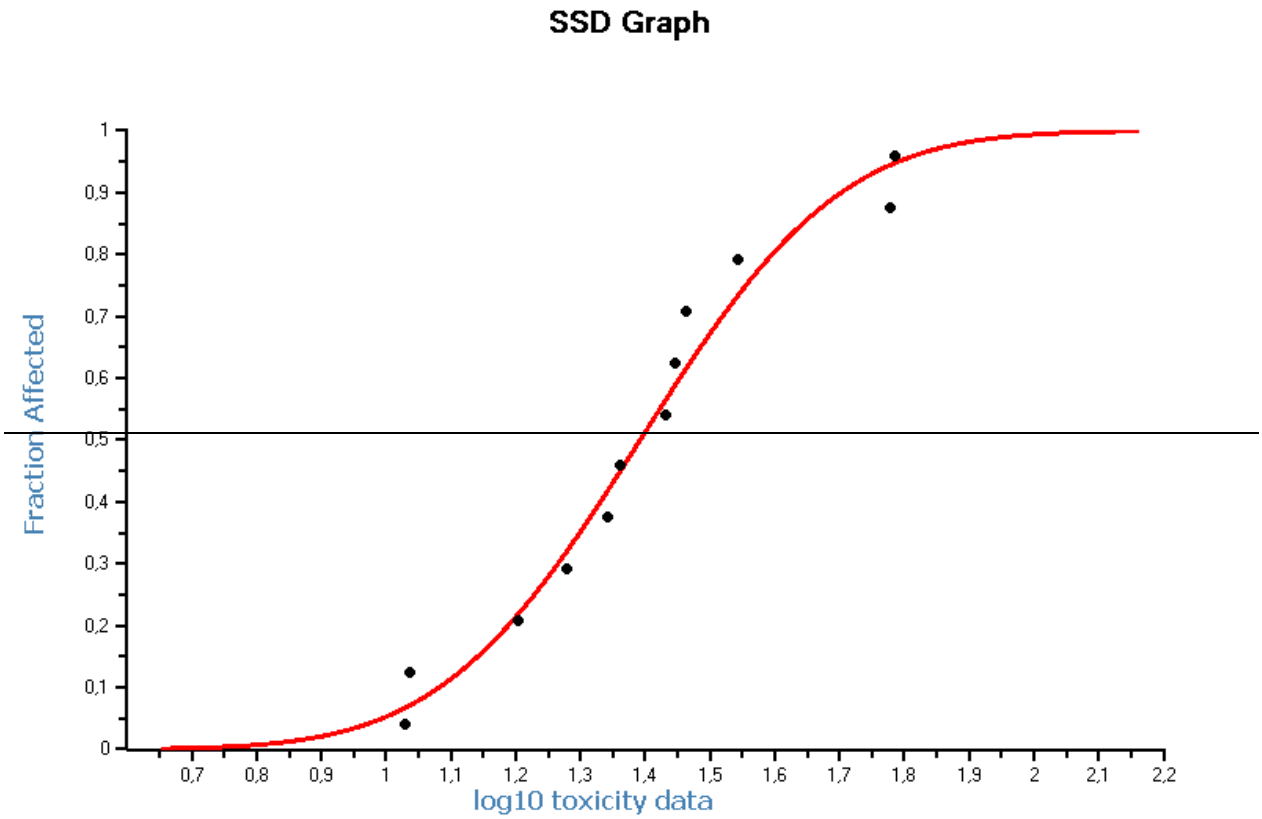
Table B.9.4-02: Acute toxicity of chlorothalonil to fish and amphibian larvae

Test species	EU agreed endpoints
Fish	
Oncorhynchus mykiss	LC_{50} = 0.022 mg/L*
Cyprinus carpio	LC_{50} = 0.060 mg/L
Cyprinodon variegatus	96-h LC_{50} = 0.028 mg/L
Galaxias maculatus	96-h LC_{50} = 0.016 mg/L
Galaxias truttaceus	96-h LC_{50} = 0.019 mg/L
Galaxias auratus	96-h LC_{50} = 0.029 mg/L
Pimephales promelas	96-h LC_{50} = 0.023 mg/L
Gasterosteus aculeatus	96-h LC_{50} = 0.027 mg/L
Pagrus major	96-h LC_{50} = 0.035 mg/L
Fundulus heteroclitus	96-h LC_{50} = 0.061 mg/L
Amphibia	
Xenopus laevis	96-h LC_{50} = 0.0109 mg/L**
Spea multiplicata	96-h LC50 = 0.0107 mg/L

*geometric mean of three values: 0.017, 0.0171 en 0.039 mg/L

**geometric mean of two values: 0.0082 and 0.0144 mg/L

To refine the assessment at higher tiers in first instance the fish and amphibian acute toxicity data were combined by the RMS in an aquatic vertebrate SSD, calculated using ETX 2.1 (Van Vlaardingen et al., 2004). ~~Following the EFSA guidance, expert judgment is required as to whether to construct a single SSD for aquatic vertebrates. The fish and amphibian data, when combined fit the model and are clearly part of the same distribution and so it is considered appropriate.~~

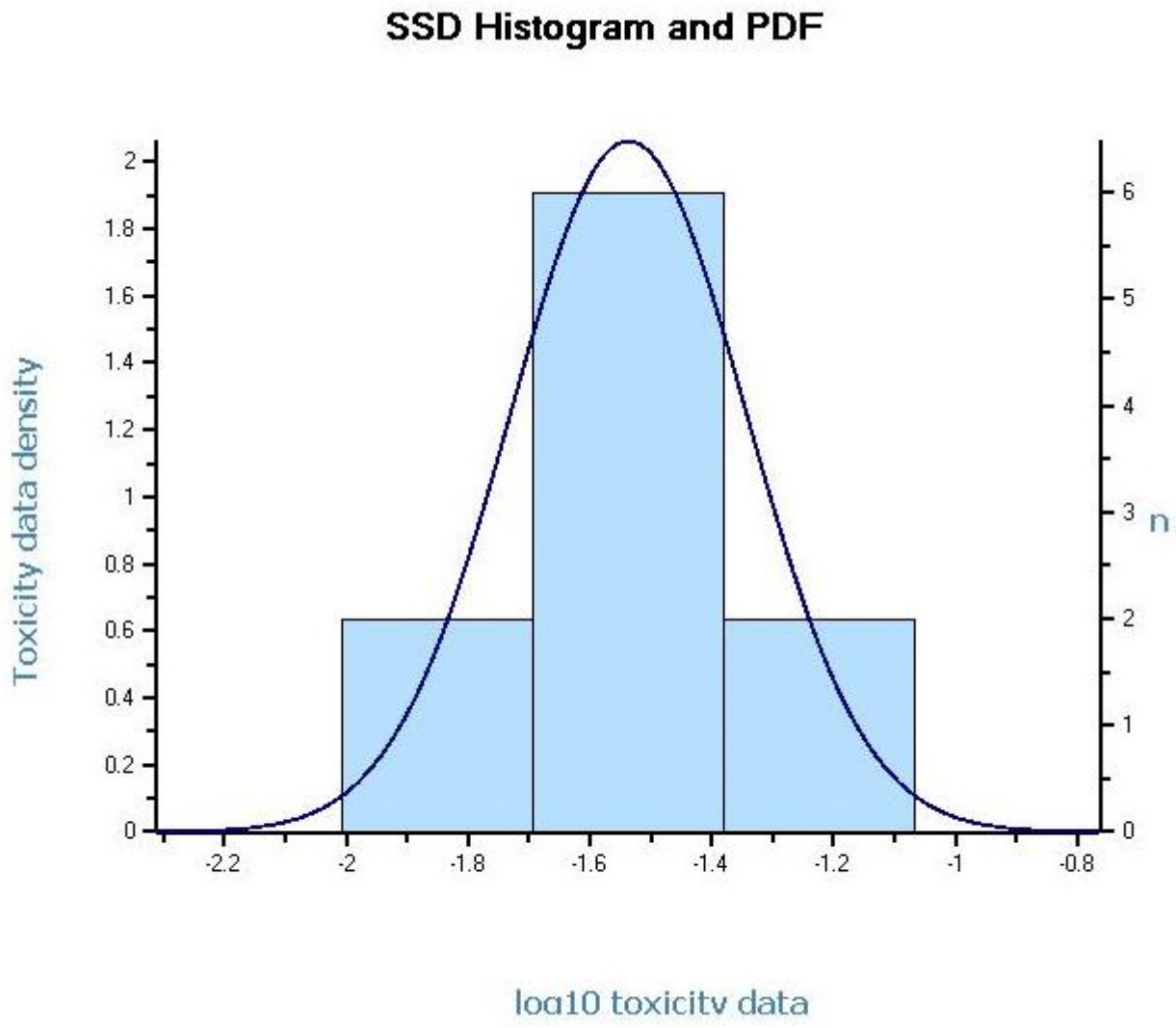


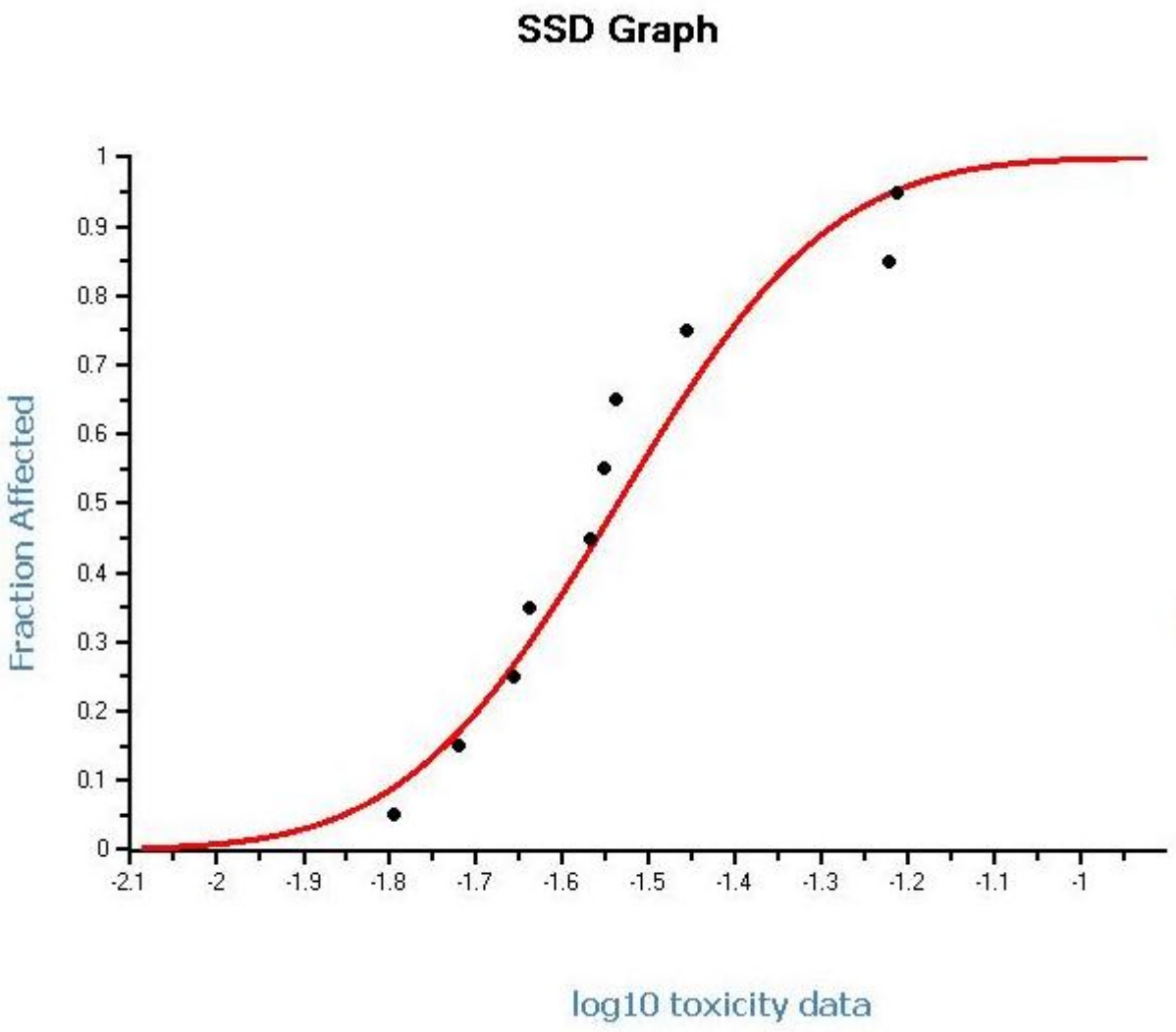
The resulting median HC5 was 9.6 µg/L (95% CI 4.66 -12.73).

However, this approach was discussed during Pesticides Peer review Meeting 165 (18-22 September 2017 and the experts considered that since from the available data amphibians appear to be more sensitive than fish, the realted endpoints should not be used for the SSD. Overall, the experts agreed not to use them in the same distribution, since there is a general lack of knowledge on the appropriateness of this approach. Hence, the acute risk assessments for fish and amphibians are done separately.

Refinement of the acute risk assessment for fish

There are valid acute endpoints from 10 different fish species available to apply the SSD approach and to calculate an HC5 value, using ETX 2.1 (Van Vlaardingen et al., 2004). The SSD histogram, curve and statistics are presented below.


SSD Histogram and PDF
Toxicity data density
n
log10 toxicity data
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
6
5
4
3
2
1
0
-2.2
-2
-1.8
-1.6
-1.4
-1.2
-1
-0.8


SSD Graph
Fraction Affected
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-2.1
-2
-1.9
-1.8
-1.7
-1.6
-1.5
-1.4
-1.3
-1.2
-1.1
-1
log10 toxicity data

Toxicity data

Anderson-Darling test for normality

Sign. level	Critical	Normal?
0.1	0.631	Accepted
0.05	0.752	Accepted
0.025	0.873	Accepted
0.01	1.035	Accepted

AD Statistic: 4.48E-1
n: 10

Note: below n=8, this test may not perform well.

Kolmogorov-Smirnov test for normality

Sign. level	Critical	Normal?
0.1	0.819	Accepted
0.05	0.895	Accepted
0.025	0.995	Accepted
0.01	1.035	Accepted

KS Statistic: 6.92E-1
n: 10

Note: below n=20, this test may not perform well.

Cramer von Mises test for normality

Sign. level	Critical	Normal?
0.1	0.104	Accepted
0.05	0.126	Accepted
0.025	0.148	Accepted
0.01	0.179	Accepted

CM Statistic: 5.54E-2
n: 10

Note: below n=20, this test may not perform well.

Exposure data

Anderson-Darling test for normality

Sign. level	Critical	Normal?
0.1	0.631	
0.05	0.752	
0.025	0.873	
0.01	1.035	

AD Statistic:
n:

Note: below n=8, this test may not perform well.

Parameters of the normal distribution

Name	Value	Description
mean	-1.54E0	mean of the log toxicity values
s.d.	1.93E-1	sample standard deviation
n	1.00E1	sample size

HC5 results

Name	Value	log10(Value)	Description
LL HC5	7.951E-3	-2.100E0	lower estimate of the HC5
HC5	1.363E-2	-1.866E0	median estimate of the HC5
UL HC5	1.848E-2	-1.733E0	upper estimate of the HC5
sprHC5	2.324E0	3.663E-1	spread of the HC5 estimate

FA At HC5 results

Name	Value	Description
FA lower	0.61	5% confidence limit of the FA at standardised median logHC5
FA median	5.00	50% confidence limit of the FA at standardised median logHC5
FA upper	20.04	95% confidence limit of the FA at standardised median logHC5

HC50 results

Name	Value	log10(Value)	Description
LL HC50	2.246E-2	-1.649E0	lower estimate of the HC50
HC50	2.908E-2	-1.536E0	median estimate of the HC50
UL HC50	3.764E-2	-1.424E0	upper estimate of the HC50
sprHC50	1.676E0	2.243E-1	spread of the HC50 estimate

FA At HC50 results

Name	Value	Description
FA lower	30.15	5% confidence limit of the FA at standardised median logHC50
FA median	50.00	50% confidence limit of the FA at standardised median logHC50
FA upper	69.85	95% confidence limit of the FA at standardised median logHC50

As can be seen from the tables above the HC5 is 13.6 µg a.s./L and the distribution is accepted at all levels. The HC_5 can be used to derive a concentration at which the acute risk to fish is acceptable, by applying an assessment factor (AF) of 9 as recommended in the EFSA Guidance Document on Aquatic Risk Assessment (EFSA, 2013) [16], resulting in an acute RAC of 1.5 µg a.s./L.

[16] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 186 pp. doi:10.2903/j.efsa.2013.3290.

Consequently, FOCUS Step 3 PEC_{sw} for all application scenarios have been compared with the RAC of 1.5 µg a.s./L. For those scenarios that have PEC values above 1.5 µg a.s./L, additional comparisons using FOCUS Step 4 were performed, as shown in the tables below.

Table B.9.4-03 **Acute toxicity-exposure ratios for chlorothalonil resulting from the use on spring- and winter cereals and tomatoes based on the acute HC5 for fish (with a SF of 9). Step 3.**

Scenario	Endpoint	PEC_{sw} (FOCUS Step 3)*		TER (trigger is 9)
		Scenario	µg/L	
Spring cereals, 1 x 750 g a.s./ha	HC5 = 0.0136 mg a.s./L (= 13.6 µg a.s./L)	D1 Ditch	4.80	**2.83**
		D1 Stream	4.20	**3.24**
		D3 Ditch	4.76	**2.86**
		D4 Pond	0.164	82.9
		D4 Stream	4.09	**3.32**
		D5 Pond	0.164	82.9
		D5 Stream	4.42	**3.08**
		R4 Stream	3.14	**4.33**
Spring cereals, 2 x 750 g a.s./ha	HC5 = 0.0136 mg a.s./L (= 13.6 µg a.s./L)	D1 Ditch	5.93	**2.29**
		D1 Stream	3.63	**3.75**
		D3 Ditch	4.16	**3.27**
		D4 Pond	0.229	59.4
		D4 Stream	3.55	**3.83**
		D5 Pond	0.231	58.9
		D5 Stream	3.83	**3.55**
		R4 Stream	5.07	**2.68**
Winter cereals, 1 x 750 g a.s./ha	HC5 = 0.0136 mg a.s./L (= 13.6 µg a.s./L)	D1 Ditch	4.76	**2.86**
		D1 Stream	3.70	**3.68**
		D2 Ditch	4.78	**2.85**
		D2Stream	3.95	**3.44**
		D3 Ditch	4.74	**2.87**
		D4 Pond	0.164	82.9
		D4 Stream	3.51	**3.87**
		D5 Pond	0.164	82.9
		D5 Stream	3.79	**3.59**
		D6 Ditch	4.79	**2.84**
		R1 Pond	0.194	70.1
		R1 Stream	3.14	**4.33**
		R3 Stream	4.39	**3.10**
		R4 Stream	3.13	**4.35**
		D1 Ditch	4.19	**3.25**
		D1 Stream	3.54	**3.84**
		D2 Ditch	4.18	**3.25**
		D2Stream	3.42	**3.98**

Winter cereals, 2 x 750 g a.s./ha	HC5 = 0.0136 mg a.s./L (= 13.6 µg a.s./L)	D3 Ditch	4.15	**3.28**
		D4 Pond	0.203	67.0
		D4 Stream	3.13	**4.35**
		D5 Pond	0.232	58.6
		D5 Stream	3.62	**3.76**
		D6 Ditch	4.32	**3.15**
		R1 Pond	0.235	57.9
		R1 Stream	2.82	**4.82**
		R3 Stream	3.82	**3.56**
		R4 Stream	2.71	**5.02**
Tomatoes, 1 x 1000 g a.s./ha	HC5 = 0.0136 mg a.s./L (= 13.6 µg a.s./L)	D6 Ditch	6.29	**2.16**
		R2 Stream	5.61	**2.42**
		R3 Stream	5.90	**2.31**
		R4 Stream	5.42	**2.51**

From table B.9.4-03 it appears that for all scenarios for the different uses, except the pond scenarios, the TER is below the trigger value of 9. Hence, a further refinement is necessary.

As a refinement the notfier has submitted FOCUS step 4 values. Risk mitigation measures are proposed (Run-off mitigation in combination with spray-drift buffers). In table B.9.4-04 - 06 the FOCUS step 4 PECsw values are presented for the different uses and scenarios. The acute RAC for fish is 1.5 µg a.s./L . FOCUS Step 4 values above this value are in bold.

Table B.9.4-04: Comparison of exposure scenarios following application of chlorothalonil to spring cereals at FOCUS Step 4 to RAC of 1.5 µg a.s./L

Crop (Use pattern)	**Scenario**	**Water body**	**FOCUS Step 4 PEC_{SW} [µg/L]**		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Spring cereals 1 x 750 g a.s./ha BBCH 30	D1	Ditch	0.690	-	-
	D1	Stream	0.814	-	-
	D3	Ditch	0.684	-	-
	D4	Pond	-	-	-
	D4	Stream	0.793	-	-
	D5	Pond	-	-	-
	D5	Stream	0.857	-	-
	R4	Stream	0.608	-	-
Spring cereals 2 x 750 g a.s./ha BBCH 30	D1	Ditch	0.786	-	-
	D1	Stream	0.667	-	-
	D3	Ditch	0.561	-	-
	D4	Pond	-	-	-
	D4	Stream	0.651	-	-

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
	D5	Pond	-	-	-
	D5	Stream	0.702	-	-
	R4	Stream	**5.07**	**2.28**	1.19

PEC values in bold are greater than the RAC of 1.5 µg/L

Table B.9.4-05: Comparison of exposure scenarios following application of chlorothalonil to winter cereals at FOCUS Step 4 to RAC of 1.5 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Winter cereals 1 x 750 g a.s./ha BBCH 30	D1	Ditch	0.684	-	-
	D1	Stream	0.717	-	-
	D2	Ditch	0.687	-	-
	D2	Stream	0.766	-	-
	D3	Ditch	0.681	-	-
	D4	Pond	-	-	-
	D4	Stream	0.679	-	-
	D5	Pond	-	-	-
	D5	Stream	0.734	-	-
	D6	Ditch	0.688	-	-
	R1	Pond	-	-	-
	R1	Stream	**2.82**	1.28	-
	R3	Stream	0.851	-	-
	R4	Stream	0.606	-	-
Winter cereals 2 x 750 g a.s./ha BBCH 30	D1	Ditch	0.565	-	-
	D1	Stream	0.649	-	-
	D2	Ditch	0.563	-	-
	D2	Stream	0.627	-	-
	D3	Ditch	0.559	-	-
	D4	Pond	-	-	-
	D4	Stream	0.575	-	-
	D5	Pond	-	-	-
	D5	Stream	0.664	-	-
	D6	Ditch	0.582	-	-
	R1	Pond	-	-	-
	R1	Stream	**2.82**	1.28	-
	R3	Stream	**3.36**	**1.53**	0.804
	R4	Stream	**1.63**	0.736	-

PEC values in bold are greater than the RAC of 1.5 µg/L

Table B.9.4-06: Comparison of exposure scenarios following application of chlorothalonil to tomatoes at FOCUS Step 4 to RAC of 1.5 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Tomatoes 1 x 1000 g a.s./ha BBCH 51	D6	Ditch	0.904	-	-
	R2	Stream	**1.09**	1.09	-
	R3	Stream	**1.17**	1.14	-
	R4	Stream	**5.42**	**2.46**	1.29

PEC values in bold are greater than the RAC of 1.5 µg/L

The resulting comparison of FOCUS Step 3 and 4 PEC_{sw} values to the RAC of 1.5 µg/L indicate that the acute risk of chlorothalonil to fish is acceptable following the use of A14111B according to the proposed use pattern with consideration given to appropriate mitigation requirements as presented in Table B.9.4-07.

Table B.9.4-07: Mitigation requirements for acute risk to fish

Crop	Appl. Rate (g/ha)	No. of appl.	Scenario									
			D1	D2	D3	D4	D5	D6	R1	R2	R3	R4
Spring cereals	750	1	10 m SD		10 m SD	10 m SD	10 m SD					10 m SD
		2	10 m SD		10 m SD	10 m SD	10 m SD					20 m SD with 80% RO
Winter cereals	750	1	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD with 60% RO		10 m SD	10 m SD
		2	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD with 60% RO		20 m SD with 80% RO	10 m SD with 60% RO
Tomatoes	1000	1						10 m SD		20 m SD with 80% RO	20 m SD with 80% RO	20 m SD with 80% RO

A grey field means that the scenario is not relevant for this crop group
RO = run-off mitigation; SD = spray drift buffer
~~>M = mitigation greater than 80% run-off + 20 m spray buffer is required~~

The notifier proposed to refine the acute rish to fish for the R4 stream scenario based upon a WoE and the results of the pulsed dose study:

"The only scenario which does not achieve acceptable mitigation is R4 stream in spring cereals tomatoes, where the PECs of 1.19 and 1.29 µg/L exceed the RAC of 1.07 µg/L, but this is an extremely minor exceedance and the assessment is extremely conservative, as discussed below.

Following the EFSA Aquatic Guidance, it is possible to demonstrate acceptable acute risk, the most sensitive endpoint being acute risk to fish. As prescribed, this risk assessment is extremely precautionary, it compares peak exposure concentrations to endpoints derived from laboratory studies where concentrations were maintained. In natural aquatic environments chlorothalonil dissipates extremely rapidly with DT50 values measured in hours and so exposure in these laboratory studies, designed to estimate hazard is not at all environmentally realistic and will likely overestimate risk. Since chlorothalonil was approved for Annex I inclusion, and thus deemed safe to use, the risk presented by chlorothalonil has decreased as use rates have reduced and mitigation measures increased. The lack of incident reporting in EU of fish kills following use of chlorothalonil, whilst it cannot be used in any definitive way, supports the conclusion that it presents negligible acute risk to fish.

*This conclusion is strongly supported by the results of a field study with chlorothalonil (**Ernst et al., 1991**), submitted previously. Whilst the study was not designed to simulate the specific scenarios used for risk assessment within the EU, it adds to the weight of evidence for negligible effects at worst-case PECs.*
The field study was conducted using a small freshwater pond (2000 m^2 x 0.5 m mean depth) on Prince Edward Island, Canada. Three direct applications of formulated chlorothalonil at a rate of 875 g ai/ha were made at weekly intervals to the surface of the pond. Each application was equivalent to a nominal concentration of 175 µg/L, evenly distributed throughout the water column, a concentration over 100x the RACacsw considered here. Measured concentrations sampled just below the water surface immediately after each treatment ranged from 120 - 2900 µg/L, indicating some organisms may have been exposed to concentrations much higher than nominal.
The dissipation rate of chlorothalonil in the pond is not readily established due to lack of further sampling and there being an inflow and outflow in the pond corresponding to approximately 2 pond volumes/day. Nevertheless, it is possible to say that the exposure would be fairly representative of that which would happen even in a static system, as the environmental fate data shows that dissipation would occur through degradation at a rate faster than any dissipation in this system due to dilution. At the treatment rate of nominally 175 µg/L, exposure is well above that proposed from worst-case modelling scenarios and even well above acute effect levels for fish in laboratory studies.
One year-old rainbow trout were present in cages in the pond prior to and during the three applications. Despite the nominal concentration being some 5 times higher than laboratory LC_{50} values in water alone and over twice the laboratory sediment-water LC_{50}, there were no mortalities.

Within the same study, caged sticklebacks, another species with a similar sensitivity to chlorothalonil, were included. These showed partial mortality of 37%. However, these were exposed in floating cages at the surface, where exposure was very extreme (up to 2900 µg/L). Furthermore, there was no assessment of the effect of handling control cages which may be expected to contribute to stress and subsequent mortality.

When reviewed previously for Annex 1 the conclusion was "Lack of mortality in rainbow trout is surprising in view of laboratory LC50. A likely reason is the difference in water parameters between lab and field". This is certainly the case and it emphasiszes the conservatism of the current assessment

If WoE, and lack of evidence of any fish moratlity following use at much higher rates alone are insufficient to indicate acceptable acute risk to fish, data are now available to confirm it from the pulsed dose study done to investigate chronic risk to fish (K-CA IIA 8.2.2-01). This study was done to investigate chronic risk to fish from multiple pulses which exceeded the chronic RAC of 0.14 µg/L, which occurs in many scenarios. The RAC for acute risk to fish is only exceeded in the spring cereals and tomato R4 scenarios and the exceedance of the RAC of 1.07 µg/L is minor, only reaching a peak of 1.19 µg/L in spring cereals and 1.29 µg/L respectively. Fathead minnows are of similar sensitivity to all the fish species tested with a 96 h LC50 of 23 µg/L. The pulsed dose study exposed them to concentrations above the RAC with no mortalities or indeed any symptoms of toxicity, thus the acute risk to fish is acceptable".

Remark by RMS

The RMS is of opinion that the study by Ernst et al. 1991 cannot be used as concentrations were only measured just below the water surface, resulting in stickleback mortality. Moreover, regarding the observed difference in fish response between the field and lab study, the following was stated in the DAR (2000) of original approval: *Reduction of exposure through physical and chemical processes is the likely reason for this effect* (e.g. *The turn-over rate of the pond was 1.3 - 2.8 times per day, which is reflected in the high concentrations at 30 m downstream after 25 minutes (A small brook was flowing through the pond. Chlorothalonil did not reach the sediment in 24h… System already contained chlorothalonil, and possibly deltamethrin had been used in previous years*). Therefore, the RMS is of opinon that the Ernst et al. 1991 study cannot be used as a WoE. However the RMS agrees that based on the results of the pulsed dose study with fathead minnows (a species which is of similar sensitivity as the most sensitive fish species (*Oncorhynchus mykiss* and *Galaxias sp.*, see Table B.9.4-02)) in which no effects were observed at concentrations well above the RAC of 1.07 µg/L after 6, 16, 26 and 24 hours (See Table B.9.4-11 below and study CA 8.2.2/01 in the CA document) the acute risk for fish for the R4 scenario can be considered acceptable, considering the fact that the FOCUS Step 4 PEC_{sw} are close to RAC of 1.07 µg/L.

Refinement of the acute risk assessment for amphibians

The risk assessment for amphibians will be performed using the scheme for aquatic organisms, since a specific scheme is not available, although the protection goal is unknown. It is also noted that the terrestrial life stage of amphibians is not covered by this risk assessment.

The endpoint for amphibians can be refined by taking the geometric mean of the two values which are available for *Xenopus laevis*. The two values are 0.0082 and 0.0144 mg a.i./L and the geoemtric mean is 0.0109 mg a.i./L (see also table B.9.4-02). Also an endpoint is available for the amphibian species *Spea multiplicata*. This value is 0.0107 mg a.i./L. Hence, the values for the two species are practically the same and for that reason it has no sense to take the geometric mean of the two values for the two species. Hence, the acute LC50 value of 0.0107 mg a.i./L will be used for the acute risk assessment.

Table B.9.4-08 Acute toxicity-exposure ratios for chlorothalonil resulting from the use on spring- and winter cereals and tomatoes based on the acute LC50 value for amphibians of 10.7 µg a.s./L with a safety factor of 100. Step 3.

Scenario	Endpoint	PEC_{sw} (FOCUS Step 3)*		TER (trigger is 100)
		Scenario	µg/L	
Spring cereals, 1 x 750 g a.s./ha	LC50 = 10.7 µg a.s./L	D1 Ditch	4.80	**2.2**
		D1 Stream	4.20	**2.5**
		D3 Ditch	4.76	**2.2**
		D4 Pond	0.164	**65.2**
		D4 Stream	4.09	**2.6**
		D5 Pond	0.164	**65.2**
		D5 Stream	4.42	**2.4**
		R4 Stream	3.14	**3.4**
Spring cereals, 2 x 750 g a.s./ha	LC50 = 10.7 µg a.s./L	D1 Ditch	5.93	**1.8**
		D1 Stream	3.63	**2.9**
		D3 Ditch	4.16	**2.6**
		D4 Pond	0.229	**46.7**
		D4 Stream	3.55	**3.0**
		D5 Pond	0.231	**46.3**
		D5 Stream	3.83	**2.8**
		R4 Stream	5.07	**2.1**
Winter cereals, 1 x 750 g a.s./ha	LC50 = 10.7 µg a.s./L	D1 Ditch	4.76	**2.2**
		D1 Stream	3.70	**2.9**
		D2 Ditch	4.78	**2.2**
		D2Stream	3.95	**2.7**
		D3 Ditch	4.74	**2.3**
		D4 Pond	0.164	**65.2**
		D4 Stream	3.51	**3.0**
		D5 Pond	0.164	**65.2**
		D5 Stream	3.79	**2.8**
		D6 Ditch	4.79	**2.2**
		R1 Pond	0.194	**55.2**
		R1 Stream	3.14	**3.4**
		R3 Stream	4.39	**2.4**
		R4 Stream	3.13	**3.4**

Winter cereals, 2 x 750 g a.s./ha	LC50 = 10.7 µg a.s./L	D1 Ditch	4.19	**2.6**
		D1 Stream	3.54	**3.0**
		D2 Ditch	4.18	**2.6**
		D2Stream	3.42	**3.1**
		D3 Ditch	4.15	**2.6**
		D4 Pond	0.203	**52.7**
		D4 Stream	3.13	**3.4**
		D5 Pond	0.232	**46.1**
		D5 Stream	3.62	**3.0**
		D6 Ditch	4.32	**2.5**
		R1 Pond	0.235	**45.5**
		R1 Stream	2.82	**3.8**
		R3 Stream	3.82	**2.8**
		R4 Stream	2.71	**3.9**
Tomatoes, 1 x 1000 g a.s./ha	LC50 = 10.7 µg a.s./L	D6 Ditch	6.29	**1.70**
		R2 Stream	5.61	**1.91**
		R3 Stream	5.90	**1.81**
		R4 Stream	5.42	**1.97**

From table B.9.4-08 it appears that for all scenarios for the different uses the TER is below the trigger value of 100. Hence, a further refinement of the actue risk to amphibians is necessary.

As a refinement the notfier has submitted FOCUS step 4 values. Risk mitigation measures are proposed (Run-off mitigation in combination with spray-drift buffers). In table B.9.4-09 - 11 the FOCUS step 4 PECsw values are presented for the different uses and scenarios. The acute RAC for amphibians is 0.107 µg a.s./L . FOCUS Step 4 values above this value are in bold.

Table B.9.4-09: Comparison of exposure scenarios following application of chlorothalonil to spring cereals at FOCUS Step 4 to RAC of 0.107 µg a.s./L

Crop (Use pattern)	**Scenario**	**Water body**	**FOCUS Step 4 PEC_{sw} [µg/L]**		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Spring cereals 1 x 750 g a.s./ha BBCH 30	D1	Ditch	**0.690**	**0.690**	**0.359**
	D1	Stream	**0.814**	**0.814**	**0.423**
	D3	Ditch	**0.684**	**0.684**	**0.355**
	D4	Pond	0.102	0.102	0.068
	D4	Stream	**0.793**	**0.793**	**0.412**
	D5	Pond	0.102	0.102	0.068
	D5	Stream	**0.857**	**0.857**	**0.445**
	R4	Stream	**0.608**	**0.608**	**0.316**
Spring cereals	D1	Ditch	**0.786**	**0.786**	**0.397**

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			-	60%	80%
Spray-drift buffer			10 m	10 m	20 m
2 x 750 g a.s./ha BBCH 30	D1	Stream	**0.667**	**0.667**	**0.339**
	D3	Ditch	**0.561**	**0.561**	**0.285**
	D4	Pond	**0.139**	**0.139**	0.091
	D4	Stream	**0.651**	**0.651**	**0.331**
	D5	Pond	**0.141**	**0.141**	0.092
	D5	Stream	**0.702**	**0.702**	**0.357**
	R4	Stream	**5.070**	**2.277**	**1.188**

PEC values in bold are greater than the RAC of 0.107 µg/L

Table B.9.4-10: Comparison of exposure scenarios following application of chlorothalonil to winter cereals at FOCUS Step 4 to RAC of 0.107 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			-	60%	80%
Spray-drift buffer			10 m	10 m	20 m
Winter cereals 1 x 750 g a.s./ha BBCH 30	D1	Ditch	**0.684**	**0.684**	**0.356**
	D1	Stream	**0.717**	**0.717**	**0.373**
	D2	Ditch	**0.687**	**0.687**	**0.357**
	D2	Stream	**0.766**	**0.766**	**0.398**
	D3	Ditch	**0.681**	**0.681**	**0.354**
	D4	Pond	0.102	0.102	0.068
	D4	Stream	**0.679**	**0.679**	**0.353**
	D5	Pond	0.102	0.102	0.068
	D5	Stream	**0.734**	**0.734**	**0.381**
	D6	Ditch	**0.688**	**0.688**	**0.358**
	R1	Pond	**0.144**	**0.107**	0.068
	R1	Stream	**2.819**	**1.275**	**0.667**
	R3	Stream	**0.851**	**0.851**	**0.442**
	R4	Stream	**0.606**	**0.606**	**0.315**
Winter cereals 2 x 750 g a.s./ha BBCH 30	D1	Ditch	**0.565**	**0.565**	**0.287**
	D1	Stream	**0.649**	**0.649**	**0.330**
	D2	Ditch	**0.563**	**0.563**	**0.286**
	D2	Stream	**0.627**	**0.627**	**0.319**
	D3	Ditch	**0.559**	**0.559**	**0.284**
	D4	Pond	**0.124**	**0.112**	0.081
	D4	Stream	**0.575**	**0.575**	**0.292**
	D5	Pond	**0.141**	**0.127**	0.093
	D5	Stream	**0.664**	**0.664**	**0.338**
	D6	Ditch	**0.582**	**0.569**	**0.295**
	R1	Pond	**0.157**	**0.123**	0.086

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
	R1	Stream	**2.819**	**1.275**	**0.667**
	R3	Stream	**3.355**	**1.531**	**0.804**
	R4	Stream	**1.631**	**0.736**	**0.384**

PEC values in bold are greater than the RAC of 0.107 µg/L

Table B.9.4-11: Comparison of exposure scenarios following application of chlorothalonil to tomatoes at FOCUS Step 4 to RAC of 0.107 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Tomatoes 1 x 1000 g a.s./ha BBCH 51	D6	Ditch	**0.904**	**0.904**	**0.470**
	R2	Stream	**1.087**	**1.087**	**0.565**
	R3	Stream	**1.169**	**1.143**	**0.594**
	R4	Stream	**5.419**	**2.465**	**1.292**

PEC values in bold are greater than the RAC of 0.107 µg/L

The resulting comparison of FOCUS Step 3 and 4 PEC_{sw} values to the RAC of 0.107 µg/L indicate that the acute risk of chlorothalonil to amphibians is not acceptable following the use of A14111B according to the proposed use pattern for all scenarios and all proposed risk mitigation measures. Only in the case of the pond scenarios the risk is acceptable, taking into account the proposed risk mitigation measures.

Hence, a further refinement of the acute risk to amphibians is required.

B.9.4.2.1 Refined acute risk assessment to aquatic invertebrates for chlorothalonil

The acute risk to aquatic invertebrates requires refinement. The lowest L(E)C50 for aquatic invertebrates is the value of 5 µg a.s./L (for *Crassostrea virginica)*, applied with a safety factor of 100. The first refinement is the comparison of the toxicity with FOCUS Step 3 values for the different uses.

In table B.9.4-12 FOCUS Step 3 PEC_{SW} for all application scenarios have been compared with the lowest L(E)C50 value.

Table B.9.4-12 Acute toxicity-exposure ratios for chlorothalonil resulting from the use on spring- and winter cereals and tomatoes based on the lowest acute L(E)C50 value for aquatic invertebrates of 5 µg a.s./L with a safety factor of 100. Step 3.

Scenario	Endpoint	PEC_{sw} (FOCUS Step 3)*		TER (trigger is 100)
		Scenario	µg/L	
Spring cereals, 1 x 750 g a.s./ha	LC50 = 5 µg a.s./L	D1 Ditch	4.80	**1.04**
		D1 Stream	4.20	**1.19**

		D3 Ditch	4.76	**1.05**
		D4 Pond	0.164	**30.49**
		D4 Stream	4.09	**1.22**
		D5 Pond	0.164	**30.49**
		D5 Stream	4.42	**1.13**
		R4 Stream	3.14	**1.59**
Spring cereals, 2 x 750 g a.s./ha	LC50 = 5 µg a.s./L	D1 Ditch	5.93	**0.84**
		D1 Stream	3.63	**1.38**
		D3 Ditch	4.16	**1.20**
		D4 Pond	0.229	**21.8**
		D4 Stream	3.55	**1.41**
		D5 Pond	0.231	**21.7**
		D5 Stream	3.83	**1.31**
		R4 Stream	5.07	**0.99**
Winter cereals, 1 x 750 g a.s./ha	LC50 = 5 µg a.s./L	D1 Ditch	4.76	**1.05**
		D1 Stream	3.70	**1.35**
		D2 Ditch	4.78	**1.05**
		D2Stream	3.95	**1.27**
		D3 Ditch	4.74	**1.05**
		D4 Pond	0.164	**30.49**
		D4 Stream	3.51	**1.42**
		D5 Pond	0.164	**30.49**
		D5 Stream	3.79	**1.32**
		D6 Ditch	4.79	**1.04**
		R1 Pond	0.194	**25.8**
		R1 Stream	3.14	**1.59**
		R3 Stream	4.39	**1.14**
		R4 Stream	3.13	**1.60**
Winter cereals, 2 x 750 g a.s./ha	LC50 = 5 µg a.s./L	D1 Ditch	4.19	**1.19**
		D1 Stream	3.54	**1.41**
		D2 Ditch	4.18	**1.20**
		D2Stream	3.42	**1.46**
		D3 Ditch	4.15	**1.20**
		D4 Pond	0.203	**24.6**
		D4 Stream	3.13	**1.60**
		D5 Pond	0.232	**21.6**
		D5 Stream	3.62	**1.38**
		D6 Ditch	4.32	**1.16**
		R1 Pond	0.235	**21.3**
		R1 Stream	2.82	**1.77**
		R3 Stream	3.82	**1.31**
		R4 Stream	2.71	**1.85**
Tomatoes, 1 x	LC50 = 5 µg a.s./L	D6 Ditch	6.29	**0.79**

1000 g a.s./ha		R2 Stream	5.61	**0.89**
		R3 Stream	5.90	**0.85**
		R4 Stream	5.42	**0.92**

From table B.9.4-12 it appears that for all scenarios for the different uses the TER is below the trigger value of 100. Hence, a further refinement is necessary. Two mesocoms studies are available, summarised and evaluated in the CA –document, section B.9. The risk assessment based on the results of the mesocosm studies is presented after the chronic risk assessment for aquatic (in)vertebrates below.

B.9.4.2.2 Refined risk assessment to primary producers for chlorothalonil

The risk to primary producers requires refinement. The lowest ErC50 for primary producers is the value of 13 µg a.s./L, applied with a safety factor of 10. The first refinement is the comparison of the toxicity with FOCUS Step 3 values for the different uses.

In table B.9.4-13 FOCUS Step 3 PEC_{SW} for all application scenarios have been compared with the lowest ErC50 value for primary producers.

Table B.9.4-13 Toxicity-exposure ratios for chlorothalonil resulting from the use on spring- and winter cereals and tomatoes based on the lowest ErC50 value of 13 µg a.s./L for primary producers (with a SF of 10). Step 3.

Scenario	Endpoint	PEC_{sw} (FOCUS Step 3)*		TER (trigger is 10)
		Scenario	µg/L	
Spring cereals, 1 x 750 g a.s./ha	ErC50 = 13 µg a.s./L	D1 Ditch	4.80	**2.71**
		D1 Stream	4.20	**3.10**
		D3 Ditch	4.76	**2.73**
		D4 Pond	0.164	79.27
		D4 Stream	4.09	**3.18**
		D5 Pond	0.164	79.27
		D5 Stream	4.42	**2.94**
		R4 Stream	3.14	**4.14**
Spring cereals, 2 x 750 g a.s./ha	ErC50 = 13 µg a.s./L	D1 Ditch	5.93	**2.19**
		D1 Stream	3.63	**3.58**
		D3 Ditch	4.16	**3.13**
		D4 Pond	0.229	56.8
		D4 Stream	3.55	**3.66**
		D5 Pond	0.231	56.3
		D5 Stream	3.83	**3.39**
		R4 Stream	5.07	**2.56**
Winter cereals, 1 x 750 g a.s./ha	ErC50 = 13 µg a.s./L	D1 Ditch	4.76	**2.73**
		D1 Stream	3.70	**3.51**

		D2 Ditch	4.78	**2.72**
		D2 Stream	3.95	**3.29**
		D3 Ditch	4.74	**2.74**
		D4 Pond	0.164	79.3
		D4 Stream	3.51	**3.70**
		D5 Pond	0.164	79.3
		D5 Stream	3.79	**3.43**
		D6 Ditch	4.79	**2.71**
		R1 Pond	0.194	67.0
		R1 Stream	3.14	**4.14**
		R3 Stream	4.39	**2.96**
		R4 Stream	3.13	**4.15**
Winter cereals, 2 x 750 g a.s./ha	ErC50 = 13 µg a.s./L	D1 Ditch	4.19	**3.10**
		D1 Stream	3.54	**3.67**
		D2 Ditch	4.18	**3.11**
		D2Stream	3.42	**3.80**
		D3 Ditch	4.15	**3.13**
		D4 Pond	0.203	64.0
		D4 Stream	3.13	**4.15**
		D5 Pond	0.232	56.0
		D5 Stream	3.62	**3.59**
		D6 Ditch	4.32	**3.01**
		R1 Pond	0.235	55.3
		R1 Stream	2.82	**4.61**
		R3 Stream	3.82	**3.40**
		R4 Stream	2.71	**4.80**
Tomatoes, 1 x 1000 g a.s./ha	ErC50 = 13 µg a.s./L	D6 Ditch	6.29	**2.07**
		R2 Stream	5.61	**2.32**
		R3 Stream	5.90	**2.20**
		R4 Stream	5.42	**2.40**

From table B.9.4-13 it appears that for all scenarios for the different uses, except the pond scenarios, the TER is below the trigger value of 10. Hence, a further refinement is necessary. Two mesocoms studies are available, summarised and evaluated in the CA –document, section B.9. The risk assessment based on the results of the mesocosm studies is presented after the chronic risk assessment for aquatic (in)vertebrates below.

B.9.4.3 Long-term risk

First tier risk assessment based on FOCUS Step 2 exposure values

The chronic Toxicity-Exposure Ratios (TERs) for the use in winter- and spring cereals and tomatoes are calculated with FOCUS Step 2 values. The TERs are presented in the table below.

Chronic Toxicity-Exposure Ratios for aquatic organisms based on max FOCUS Step 2 PEC values

Species	**L(E)C_{50}** or **NOEC [µg as/L]**	**Cereals** **Max FOCUS Step 2**	**Tomatoes** **Max FOCUS Step 2**
		18.3 µg as/L 0.237 mg/kg sed	9.20 µg as/L 0.098 mg/kg sed
Chronic			
Fish	1.4	**0.07**	**0.15**
Amphibians	-*	**-**	**-**
Invertebrates	0.40	**0.022**	**0.04**
Chironomus riparius	40	**2.19**	**4.34**
Hyalella azteca	7.5 mg as/kg sed	31.6	76.5

*No chronic endpoint for amphibians is available. A LAGDA (Larval Amphibian Growth and Development Assay) is requested. Reference is made the section below regarding the chronic risk to amphibians and to the endocrine disruption section.

Based on FOCUS Step 2 PECsw values, the chronic trigger (10) for aquatic vertebrates (fish and amphibians) and aquatic invertebrates (including Chironomus) are not met for all uses. Only the trigger for *Hyalella azteca* is met. Further refinement of the chronic risk for aquatic (in)vertebrates is necessary.

B.9.4.3.1 Refined Chronic risk to aquatic vertebrates (fish and amphibians)

Fish

For fish, the lowest NOEC is a 'lower than' value. It is the NOEC from a fish short term reproduction assay; the 21-d NOEC is <0.078 µg a.s./L. However, according to OECD 229, this study was not designed for risk assessment. This FSTRA test guideline states "These analyses will inform whether further longer term testing for adverse effects (namely, survival, development, growth and reproduction) is required for the chemical, rather than for use in risk assessment." In this case there is uncertainty as to the long-term effects, based on the endpoint eggs per surviving female per day. This endpoint is not originally reported in the FFLC study of Shults et al. (1980). Therefore the notifier did an additional exercise and performed a statistical analysis of this endpoint regarding the latter study. Both the NOEC and the EC10 were determined. For the analysis reference is made to the CA-document, section B.9.2.2. The lowest NOEC/EC10 value was 1.4 µg a.s./L.This endpoint is used for

risk assessment.

The applicant has also submitted refined PECsw values, based on 7 days time-weighted-average values. However, this refinement is not accepted at the moment according to the Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015). It was agreed that until further guidance on reciprocity and latency of effects are available, then the use of TWA approaches are unlikely to be sufficiently robust to be used in regulatory risk assessment.

In table B.9.4-14 FOCUS Step 3 PEC_{SW} for all application scenarios have been compared with the NOEC of 1.4 µg a.s./L.

Table B.9.4-14 Toxicity-exposure ratios for chlorothalonil resulting from the use on spring- and winter cereals and tomatoes based on the NOEC value of 1.4 µg a.s./L for fish (with a SF of 10). Step 3.

Scenario	Endpoint	PEC_{sw} (FOCUS Step 3)*		TER (trigger is 10)
		Scenario	µg/L	
Spring cereals, 1 x 750 g a.s./ha	NOEC = 1.4 µg a.s./L	D1 Ditch	4.80	**0.29**
		D1 Stream	4.20	**0.33**
		D3 Ditch	4.76	**0.29**
		D4 Pond	0.164	**8.54**
		D4 Stream	4.09	**0.34**
		D5 Pond	0.164	**8.54**
		D5 Stream	4.42	**0.32**
		R4 Stream	3.14	**0.45**
Spring cereals, 2 x 750 g a.s./ha	NOEC = 1.4 µg a.s./L	D1 Ditch	5.93	**0.27**
		D1 Stream	3.63	**0.39**
		D3 Ditch	4.16	**0.34**
		D4 Pond	0.229	**6.11**
		D4 Stream	3.55	**0.39**
		D5 Pond	0.231	**6.06**
		D5 Stream	3.83	**0.37**
		R4 Stream	5.07	**0.28**
Winter cereals, 1 x 750 g a.s./ha	NOEC = 1.4 µg a.s./L	D1 Ditch	4.76	**0.29**
		D1 Stream	3.70	**0.38**
		D2 Ditch	4.78	**0.29**
		D2Stream	3.95	**0.35**
		D3 Ditch	4.74	**0.30**
		D4 Pond	0.164	**8.54**
		D4 Stream	3.51	**0.40**
		D5 Pond	0.164	**8.54**
		D5 Stream	3.79	**0.37**
		D6 Ditch	4.79	**0.29**
		R1 Pond	0.194	**7.22**

		R1 Stream	3.14	**0.45**
		R3 Stream	4.39	**0.32**
		R4 Stream	3.13	**0.45**
Winter cereals, 2 x 750 g a.s./ha	NOEC = 1.4 µg a.s./L	D1 Ditch	4.19	**0.33**
		D1 Stream	3.54	**0.40**
		D2 Ditch	4.18	**0.33**
		D2Stream	3.42	**0.41**
		D3 Ditch	4.15	**0.34**
		D4 Pond	0.203	**6.90**
		D4 Stream	3.13	**0.45**
		D5 Pond	0.232	**6.03**
		D5 Stream	3.62	**0.39**
		D6 Ditch	4.32	**0.32**
		R1 Pond	0.235	**5.96**
		R1 Stream	2.82	**0.50**
		R3 Stream	3.82	**0.37**
		R4 Stream	2.71	**0.52**
Tomatoes, 1 x 1000 g a.s./ha	NOEC = 1.4 µg a.s./L	D6 Ditch	6.29	**0.22**
		R2 Stream	5.61	**0.25**
		R3 Stream	5.90	**0.24**
		R4 Stream	5.42	**0.26**

From table B.9.4-14 it appears that for all scenarios for the different uses, the TERs are below the trigger value of 10.

As a refinement the notfier has submitted FOCUS step 4 values. Risk mitigation measures are proposed (Run-off mitigation in combination with spray-drift buffers). In table B.9.4-15 - 17 the FOCUS step 4 PECsw values are presented for the different uses and scenarios. The chronic RAC for fish is 0.14 µg a.s./L . FOCUS Step 4 values above this value are in bold.

Table B.9.4-15: Comparison of exposure scenarios following application of chlorothalonil to spring cereals at FOCUS Step 4 to RAC of 0.14 µg a.s./L

Crop (Use pattern)	**Scenario**	**Water body**	**FOCUS Step 4 PEC_{sw} [µg/L]**		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Spring cereals 1 x 750 g a.s./ha BBCH 30	D1	Ditch	**0.690**	**0.690**	**0.359**
	D1	Stream	**0.814**	**0.814**	**0.423**
	D3	Ditch	**0.684**	**0.684**	**0.355**
	D4	Pond	0.102	0.102	0.068
	D4	Stream	**0.793**	**0.793**	**0.412**
	D5	Pond	0.102	0.102	0.068

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			-	60%	80%
Spray-drift buffer			10 m	10 m	20 m
Spring cereals 2 x 750 g a.s./ha BBCH 30	D5	Stream	**0.857**	**0.857**	**0.445**
	R4	Stream	**0.608**	**0.608**	**0.316**
	D1	Ditch	**0.786**	**0.786**	**0.397**
	D1	Stream	**0.667**	**0.667**	**0.339**
	D3	Ditch	**0.561**	**0.561**	**0.285**
	D4	Pond	0.139	0.139	0.091
	D4	Stream	**0.651**	**0.651**	**0.331**
	D5	Pond	**0.141**	**0.141**	0.092
	D5	Stream	**0.702**	**0.702**	**0.357**
	R4	Stream	**5.070**	**2.277**	**1.188**

PEC values in bold are greater than the RAC of 0.14 µg/L

Table B.9.4-16: Comparison of exposure scenarios following application of chlorothalonil to winter cereals at FOCUS Step 4 to RAC of 0.14 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			-	60%	80%
Spray-drift buffer			10 m	10 m	20 m
Winter cereals 1 x 750 g a.s./ha BBCH 30	D1	Ditch	**0.684**	**0.684**	**0.356**
	D1	Stream	**0.717**	**0.717**	**0.373**
	D2	Ditch	**0.687**	**0.687**	**0.357**
	D2	Stream	**0.766**	**0.766**	**0.398**
	D3	Ditch	**0.681**	**0.681**	**0.354**
	D4	Pond	0.102	0.102	0.068
	D4	Stream	**0.679**	**0.679**	**0.353**
	D5	Pond	0.102	0.102	0.068
	D5	Stream	**0.734**	**0.734**	**0.381**
	D6	Ditch	**0.688**	**0.688**	**0.358**
	R1	Pond	**0.144**	0.107	0.068
	R1	Stream	**2.819**	**1.275**	**0.667**
	R3	Stream	**0.851**	**0.851**	**0.442**
	R4	Stream	**0.606**	**0.606**	**0.315**
Winter cereals 2 x 750 g a.s./ha BBCH 30	D1	Ditch	**0.565**	**0.565**	**0.287**
	D1	Stream	**0.649**	**0.649**	**0.330**
	D2	Ditch	**0.563**	**0.563**	**0.286**
	D2	Stream	**0.627**	**0.627**	**0.319**
	D3	Ditch	**0.559**	**0.559**	**0.284**
	D4	Pond	0.124	0.112	0.081
	D4	Stream	**0.575**	**0.575**	**0.292**
	D5	Pond	**0.141**	0.127	0.093

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
	D5	Stream	**0.664**	**0.664**	**0.338**
	D6	Ditch	**0.582**	**0.569**	**0.295**
	R1	Pond	**0.157**	0.123	0.086
	R1	Stream	**2.819**	**1.275**	**0.667**
	R3	Stream	**3.355**	**1.531**	**0.804**
	R4	Stream	**1.631**	**0.736**	**0.384**

PEC values in bold are greater than the RAC of 0.14 µg/L

Table B.9.4-17: Comparison of exposure scenarios following application of chlorothalonil to tomatoes at FOCUS Step 4 to RAC of 0.14 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Tomatoes 1 x 1000 g a.s./ha BBCH 51	D6	Ditch	**0.904**	**0.904**	**0.470**
	R2	Stream	**1.087**	**1.087**	**0.565**
	R3	Stream	**1.169**	**1.143**	**0.594**
	R4	Stream	**5.419**	**2.465**	**1.292**

PEC values in bold are greater than the RAC of 0.14 µg/L

The resulting comparison of FOCUS Step 3 and 4 PEC_{sw} values to the RAC of 0.14 µg/L indicate that the chronic risk of chlorothalonil to fish is not acceptable following the use of A14111B according to the proposed use pattern for all scenarios and all proposed risk mitigation measures. Only in the case of the pond scenarios the risk is acceptable, taking into account the proposed risk mitigation measures.

Hence, a further refinement of the chronic risk to fish is required.

Refinement of the chronic risk to fish using pulsed dose study

The notifier proposes refining the chronic rish to fish based upon the pulsed dose study (See section B.8.5 for the exposure pattern of selected STEP 4 simulations):

The pulsed dose study is summarised in the CA document (See B.9.2.2 for more information) *and the nominal and actual pulses achieved are summarised below.*

Pulses in pulsed-dose FSTRA study

Treatment	*Pusle 1*	*Pulse 2*	*Pulse 3*	*Time between pulses*
1	*3.2*/**2.2*** (11 days)*	*3.8/**3.1** (11 days)*		
2	*12.7/**10.7** (26 hours)*			
3	*14.6/**12.9** (16 hours)*	*3.5/**2.2** (6 hours)*	*16.9/**10.1** (16 hours)*	*5/7 days*
4	***3.8**/3.0 (6 hours)*	*15.4/**10.1** (16 hours)*	*4.4/**2.2** (16 hours)*	*5/7 days*
5	*6.4/**5.7** (24 hours)*	*15.5/**14.1** (20 hours)*		*16 days*

** maximum measured concentration*

*** mean concentration of pulse*

There were no treatment related effects from any of the pulses on numbers of eggs/female/day nor were there any indications of effects immediately after the pulses, although the maximum measured pulse averaged 14.1 µg/L, compared to an acute fathead LC50 of 23 µg/L.

Risk assessment in EU tends to be very conservative and in the absence of any empirical evidence, the worst-case is assumed. The initial, worst-case assumption advocated in the EFSA Aquatic giuidance is that the only parameter of exposure which is relevant is the magnitude of exposure i.e. peak concentrations. However one thing that the pulsed-dose study clearly shows is that it is not only the peak concentration which drives the effect. The maximum exposure concentration was in the study was 16.9 µg/L. At this concentration, assuming the magnitude of exposure were the only driver, we would certainly have expected effects on fecundity of fatheads, given that in the FFLC study the NOEC based on fecundity was 1.4 µg/L, over an order of magnitude lower. Indeed, given the acute LC50 to fatheads of 23 µg/L, if the peak concentration drove the effct some lethal effects would have not been surprising. There were no effects on fecundity, indicating that the magnitude of expsore and the time of exposure are inmportant in determining the potential for any effects.

The EFSA guidance states that standard assessment factors should be applied in the assessment. Thus the way to approach the assessment is to compare the exposure profiles tested with modelled profiles x10. If the tested profiles show no unacceptable effects and are considered representative of the exposure profiles multiplied by 10, then the risk is acceptable.

However, there is still the consideration of how to compare the pulses in the test with the peaks in the modelling as they are not the same. The pulses in the study were designed to reach a particular concentration and maintain it for the prescribed time. Exposure in the study was very well described with samples taken allowing a good characterisation of exposure, yet it can never have the same level

of characterisation as a modelled exposure unless measurements were taken on the same schedule as the time steps in the model (hourly in FOCUS SW). The modelled exposures were "peaks" i.e. with a defined increase and decrease to a "peak" and so comparison of the modelled peaks with the (measured) pulses is not comparing like with like and is overly conservative – yet it is what you might do in the absence of any further information,. We have further information from the fish FFLC and the FSTRA study where, as discussed above, maintained concentrations gave effects at concentrations well below those peak concentrations in the pulse dose study with no effects. Thus time of exposure as well as magnitude are imporatant. Therfore a better comparison is some mean measure of exposure, such as the TWA of the exposure produced out of EPAT with the mean measured concentration in the pulsed dose study, providing that the width of the pulse is similar to or longer than the width of the peak above the RAC. This is still conservative, if it ignores exposure in the study where the concentration is increasing to or decreasing from the pulse, but it is more of a like with like comparison than comparing peaks with a mean measured pulse.

This comparison indicates that the pulsed exposures are suitably worst-case representatives of the modelled exposures. The maximum modelled single pulse was an average concentration of 1.01 µg/L (max 1.32 µg/L) for 23 hours, compared to a maximum single pulse of mean concentration 14.1 µg/L for 20 hours. The pulse in the study was over 13x the modelled concentration and whilst it was stated as being for 20 hours, this does not include the time for the concentration to increase from 1.4 µg/L to the pulse concentration and decline from the pulse concentration back to below 1.4 µg/L. This is the same for the other pulses and it would typically add 1-3 hours either side of the pulse.

Another thing to note is the number of peaks in the modelling. Typically there are only 1 or 2, with a maximum of 6. The study indicates no build up of effects from the multiple pulses tested, which had relatively short intervals relative some of those in the scenarios with multiple pulses. It is therefore likely that with an interval of a few days pulses are both toxicokinetically and toxicodynamically independent. Certainly there is no evidence to the contrary.

Overall it is possible to state the potential for effects on fish reproduction from exposure to chlorothalonil following the proposed uses of A14111B is extremely low. Chlorothalonil is very rapidly degraded under environmental concentrations, confirmed this by the problems maintaining nominal concentrations once fish were introduced into the pulsed dose study. The previous position of no long-term risk due to no long-term exposure has been validated with this further fish pulsed dose testing.
The table below shows the peaks from the different scenarios, using the FOCUS step 4 values with maximum mitigation (see above). *Peaks defined as any exceedance of the chronic RAC of 0.14 µg/L.*

Exceedances of RAC of 0.14 µg/L from FOCUS step 4

Scenario		***Peak 1***	***Peak 2***	***Peak 3***	***Peak 4***	***Peak 5***	***Peak 6***	***Time between exceedances***
Winter cereals 1 x 750								

Scenario		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Time between exceedances
D1	Ditch	0.353*/**0.250**** (43 hours)						
D1	Stream	0.350/**0.119** (1 hour)						
D2	Ditch	0.355/**0.245** (117 hours)						
D2	Stream	0.396/**0.213** (3 hours)						
D3	Ditch	0.350/**0.247** (29 hours)						
D4	Pond	No exceedance						
D4	Stream	0.286/**0.080** (2 hours)						
D5	Pond	No exceedance						
D5	Stream	0.304/**0.118** (1 hour)						
D6	Ditch	0.357/**0.2** (148 hours)						
R1	Pond	No exceedance						
R1	Stream	0.315/**0.206** (5 hours)	0.675/**0.517** (14 hours)					8 days
R3	Stream	0.441/**0.295** (7 hours)	0.189/**0.173** (18 hours)					22days
R4	Stream	0.315/**0.211** 5 hours						
Winter cereals 2 x 750								
D1	Ditch	0.283/**0.213** (37 hours)	0.286/**0.192** (11 days)					25 days
D1	Stream	0.281/**0.095** (2 hours)	0.329/**0.224** (6 hours)					27 days
D2	Ditch	0.285/**0.201** (70 hours)	0.282/**0.231** (19 hours)					12 days
D2	Stream	0.318/**0.171** (2 hours)	0.249/**0.074** (1 hour)					15 days
D3	Ditch	0.280/**0.209** (26 hours)	0.281/**0.209** (28 hours)					15 days
D4	Pond	No exceedance						
D4	Stream	0.229/**0.064** (1 hour)	0.262/**0.081** 2 hours					30 days

Scenario		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Time between exceedances
D5	Pond	No exceedance						
D5	Stream	0.244/**0.068** (1 hour)	0.336/**0.167** (2 hours)					14 days
D6	Ditch	0.286/**0.215** (5.5 days)	0.294/**0.219** (5.5 days)					8 days
R1	Pond	No exceedance						
R1	Stream	0.253/**0.177** (4 hours)	0.675/**0.517** (14 hours)	0.252/**0.166** (4 hours)	0.157/**0.123** 56 hours)	0.227/**0.195** (4 hours)		9/25/18/6 days
R3	Stream	0.353/**0.237** (7 hours)	0.355/**0.255** (8 hours)	0.826/**0.622** (28 hours)				14/9 days
R4	Stream	0.252/**0.167** (4 hours)	0.253/**0.177** (4 hours)	0.389/**0.319** (17 hours)	0.358/**0.299** (17 hours)	0.290/**0.254** (26 hours)	0.145/ **0.127** (9 h)	34/21/ 3/1/5 days
Spring cereals 1x 750								
D1	Ditch	0.358/0.226 (14 days)						
D1	Stream	0.422/0.308 (24 hours)						
D3	Ditch	0.353/0.251 (37 hours)						
D4	Pond	No exceedance						
D4	Stream	0.411/0.266 (7 hours)						
D5	Pond	No exceedance						
D5	Stream	0.444/0.299 (10 hours)						
R4	Stream	0.315/0.209 (5 hours)						
Spring cereals 2 x 750								
D1	Ditch	0.287/**0.198** (10 days)	0.396/**0.243** (15 days)					4 days
D1	Stream	0.338/**0.264** (21 hours)	0.338/**0.264** (21 hours)					13 days
D3	Ditch	0.282/**0.213** (32 hours)	0.283/**0.212** (34 hours)					12 days
D4	Pond	No exceedance						
D4	Stream	0.329/**0.228** (6 hours)	0.330/**0.228** (7hours)					14 days

Scenario		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Time between exceedances
D5	Pond	No exceedance						
D5	Stream	0.356/**0.252** (9 hours)	0.356/**0.259** (9 hours)					14 days
R4	Stream	0.253/**0.178** (4 hours)	0.970/**0.742** (16 hours)	1.20/**0.842** 15 hours	0.253/**0.178** (5 hours)			4/1/11 days
Fruiting vegetables 1 x 1000								
D6	Ditch	0.463/**0.308** (17 hours)						
R2	Stream	0.563/**0.268** (3 hours)						
R3	Stream	0.592/**0.381** (11 hours)	0.288/**0.240** (22 hours)					10 days
R4	Stream	0.421/**0.254** (6 hours)	1.32/**1.01** (23 hours)	0.460/**0.381** (20 hours)				5/6 days

** maximum measured concentration*

*** mean (TWA) concentration of peak*

The refined chronic risk assesssment for fish using the pulsed dose study was discussed during Pesticides Peer review Meeting 165 (18-22 September 2017). The following is copied from the report of that meeting:

For the refinement of the fish risk assessment, the applicant proposed a pulse exposure FSTRA study including five pulse treatments. At the meeting the RMS has further shown the effects results of each pulse treatment vs the FOCUS worst-case exposure scenarios.

It is noted that this comparison was not available in the RAR, but only presented at the meeting. Nevertheless, the experts were concerned that several scenarios were not covered. It is noted that this approach could be only used as illustrative because was not fully peer reviewed before the meeting e.g. acceptability of the EPAT.

In addition, although the continuous exposure in the standard FSTRA study was considered less relevant for setting an endpoint for RA for the tier I, it showed effects that were not observed in the pulse exposure FSTRA. However, this study design is not sufficient to definitively investigate effects, but it was considered by some experts useful to raise potential concerns. The experts noted that a pulse exposure FLCTT study could have been more appropriate. However, such kind of study was not considered feasible by some other experts because with pulse exposures, sensitive life stage may not be covered.

As a general issue for pulse exposure studies, it has to be ensured that relevant life stages are covered, latency of effects should be investigated as well as the ecological relevance of the toxicology independence of the pulses.

Overall the experts considered the refinement proposed by the applicant not appropriate to further address the chronic risk for fish.

Hence, it can be concluded that the refinement by this pulsed dose study is not appropriate. A chronic risk to fish still cannot be excluded. All FOCUS Step 4 values are above the chronic RAC for fish (1.4/10 = 0.14 µg a.s./L), except for the pond scenarios.

Amphibians

As discussed in the CA document (section 9.2.3) an Amphibian Metamorphosis Assay showed significant effect on thyroid at an exposure level of 4.9 µg/L. However, this test is not intended to set regulatory endpoints for use in risk assessment, but to indicate whether a substance may have significant effect on the endocrine system. In the case of chlorothalonil, the results of the AMA suggest that at least at doses of 4.9 µg/L, chlorothalonil shows effects on thyroid of *Xenopus*. Less significant histopathological changes were also seen at the lowest tested dose (0.61 µg/L). As a result, the RMS concludes that the lowest fish endpoint of 1.4 µg/L may not be protective of amphibians, as may be the case for some subset substances[17]. This being the case, it is necessary to determine a regulatorily acceptable endpoint for use in amphibian risk assessment. As such, a LAGDA (Larval Amphibian Growth and Development Assay) is requested. The RMS notes that such an assay has also been requested for chlorothalonil by the U.S. EPA, and therefore should be available, or become available shortly.

The notifier has indicated that no LAGDA study is available, as the US EPA has not issued a test order as of yet. As a result, no higher tier data for amphibians are immediately available. The chronic risk to amphibians was further discussed during Pesticides Peer review Meeting 165 (18-22 September 2017). The following is copied from the report of that meeting:

The RMS argued that the Amphibian Metamorphosis Assay (AMA) shows that chlorothalonil affects the thyroid in Xenopus at low exposure levels: histopathological changes were seen at the lowest test dose (0.61 µg as/L) with more significant effects at higher doses. Therefore, the lowest chronic fish endpoint of 1.4 µg a.s./L may not be protective for amphibians. A LAGDA study has been requested by the RMS but was not yet provided.

The experts discussed on whether the effects observed on amphibian should be further addressed in the RA because the current selected chronic endpoint on fish does not cover effects on amphibians. A LAGDA study could be useful to address the issue related to the chronic risk assessment for amphibians. Concerns were raised on requesting further vertebrates testing; without this test however, the chronic risk assessment for amphibians cannot be considered covered by fish. Therefore a data gap was agreed for a LAGDA study.

As a second aspect, it should be considered that the effects observed in the AMA may be relevant for considering whether the substance has potential endocrine disrupting effects. Therefore, this should be acknowledged in the EFSA conclusion.

Hence, it can be concluded that a chronic risk to amphibians still cannot be excluded. The applicant is requested to perform a LAGDA study.

B.9.4.3.2 Chronic risk to aquatic invertebrates

The chronic risk to aquatic invertebrates requires refinement. The lowest NOEC for aquatic invertebrates is the value of 0.4 µg a.s./L (for *Mysidopsis bahia)*, applied with a safety factor of 10. The first refinement is the comparison of the toxicity with FOCUS Step 3 values for the different uses.

In table B.9.4-18 FOCUS Step 3 PEC_{SW} for all application scenarios have been compared with the lowest NOEC value for aquatic invertebrates.

Table B.9.4-18 Toxicity-exposure ratios for chlorothalonil resulting from the use on spring- and winter cereals and tomatoes based on the lowest NOEC value of 0.4 µg a.s./L for aquatic invertebrates (with a SF of 10). Step 3.

Scenario	Endpoint	PEC_{sw} (FOCUS Step 3)*		TER (trigger is 10)
		Scenario	µg/L	
Spring cereals, 1 x 750 g a.s./ha	NOEC = 0.4 µg a.s./L	D1 Ditch	4.80	**0.08**
		D1 Stream	4.20	**0.10**
		D3 Ditch	4.76	**0.08**
		D4 Pond	0.164	**2.44**
		D4 Stream	4.09	**0.10**
		D5 Pond	0.164	**2.44**
		D5 Stream	4.42	**0.09**
		R4 Stream	3.14	**0.13**
Spring cereals, 2 x 750 g a.s./ha	NOEC = 0.4 µg a.s./L	D1 Ditch	5.93	**0.07**
		D1 Stream	3.63	**0.11**
		D3 Ditch	4.16	**0.10**
		D4 Pond	0.229	**1.75**
		D4 Stream	3.55	**0.11**
		D5 Pond	0.231	**1.73**
		D5 Stream	3.83	**0.10**
		R4 Stream	5.07	**0.08**
Winter cereals, 1 x 750 g a.s./ha	NOEC = 0.4 µg a.s./L	D1 Ditch	4.76	**0.08**
		D1 Stream	3.70	**0.11**
		D2 Ditch	4.78	**0.08**
		D2Stream	3.95	**0.10**
		D3 Ditch	4.74	**0.08**
		D4 Pond	0.164	**2.44**
		D4 Stream	3.51	**0.11**
		D5 Pond	0.164	**2.44**

[17] Weltje, et. al. Comparative Acute and Chronic Sensitivity of Fish and Amphibians: A Critical Review of Data.(2013) Env. Tox & Chemistry 32(5): 984-994.

		D5 Stream	3.79	**0.11**
		D6 Ditch	4.79	**0.08**
		R1 Pond	0.194	**2.06**
		R1 Stream	3.14	**0.13**
		R3 Stream	4.39	**0.09**
		R4 Stream	3.13	**0.13**
Winter cereals, 2 x 750 g a.s./ha	NOEC = 0.4 µg a.s./L	D1 Ditch	4.19	**0.10**
		D1 Stream	3.54	**0.11**
		D2 Ditch	4.18	**0.10**
		D2Stream	3.42	**0.12**
		D3 Ditch	4.15	**0.10**
		D4 Pond	0.203	**1.79**
		D4 Stream	3.13	**0.13**
		D5 Pond	0.232	**1.72**
		D5 Stream	3.62	**0.11**
		D6 Ditch	4.32	**0.09**
		R1 Pond	0.235	**1.70**
		R1 Stream	2.82	**0.14**
		R3 Stream	3.82	**0.10**
		R4 Stream	2.71	**0.15**
Tomatoes, 1 x 1000 g a.s./ha	NOEC = 0.4 µg a.s./L	D6 Ditch	6.29	**0.06**
		R2 Stream	5.61	**0.07**
		R3 Stream	5.90	**0.07**
		R4 Stream	5.42	**0.07**

From table B.9.4-18 it appears that for all scenarios for the different uses, the TER is below the trigger value of 10. Hence, a further refinement is necessary. Two mesocoms studies are available, summarised and evaluated in the CA –document, section B.9. The risk assessment based on the results of the mesocosm studies is presented below.

Refinement of the risk to aquatic invertebrates and primary producers based on mesocosm studies

Two microcosm studies are available for chlorothalonil (See CA document, sction B.9). The first study (3 applications at 7 day intervals) (Tattersfield et al., 2002), which is part of the EU-DAR, has been re-evaluated and established endpoints with a NOEC of 3 µg a.s./L (based on class 1 effects only) and a NOEAEC of 10 µg a.s./L (based on class 2- 3A effects). A second study has been submitted (Schäfers 2005, single application). The NOEC (based on class 1 effects only) from this study was found to be < 4 µg a.s./L and the endpoint based on effect class 2 effects is 4 µg a.s./L. The NOEAEC (based on class 2 – 3A effects) is 12.5 µg a.s./L.

The first study can be considered as realistic worst-case with respect to the number of applications (3) compared with the representative uses (1 application in potatoes and tomatoes and two applications in cereals). The study has been done in the UK and the applications were in June (7 days interval between the applications). In all test systems concentrations in water declined rapidly following each of the three applications. At the highest treatment level 20-30% of the nominal concentration could be detected after 24 h, while that was <10% for all other treatment levels. There was no build up of residues in the water due to repeated applications. Compared with the DT50 used for FOCUS PECsw calculations (1.9 days) the exposure in the mesocosm study seems to be not realistic worst-case. However, it must be kept in mind that the DT50 used for the model calculations comes from water/sediment studies performed in the dark.

The second study was a study with only one application. The study has been done in Germany and the application was at the end of May. The exposure in this study was more worst-case than in the first study with DT50 values depending on the initial concentration, ranging between 13.5 and 42 hours at 4 and 400 µg a.s./L. This study was discussed during Pesticides Peer review Meeting 165 (18-22 September 2017) and the experts were of the opinion that no NOEC can be derived from this study. The study is only useful to derive an ERO-RAC. The NOEAEC of 12.5 µg a.s./L together with an assessment factor of 4 can be used to determine the ERO-RAC, which is 3.1 µg a.s./L.

For the uses in potatoes and tomatoes with only one application it is proposed to use the results of the study of Schäfers with only one application. For the use in cereals (2 applications) it is proposed to take the results from the study of Tattersfield et al. into account. However, as already stated, the exposure in this study is less worst-case than in the study of Schäfers. The NOEC based on only class 1 effects from this study is 3 µg a.s./L. Normally an assessment factor of 2 is applied to this value, but due to the uncertainty about the worst-casedness of the exposure in the study it is proposed to use an assessment factof of 3 in this case. The ETO-RAC is then 1 µg a.s./L, which can be used for all uses. This value was agreed during during Pesticides Peer review Meeting 165 (18-22 September 2017).

It is not deemed appropriate to derive an ERO-RAC from the study of Tattersfield, taking into account that the exposure in the study is possibly not realistic worst-case. In that case it is not possible to extrapolate recovery of effects in the study to the real field situation.

In table B.9.4-19 the FOCUS Tier 3 PECsw is compared with the derived ETO-RAC and ERO-RAC values and TER values are presented.

Table B.9.4-19 Toxicity-exposure ratios for chlorothalonil resulting from the use on spring- and winter cereals and tomatoes based on the ETO-RAC and ERO-RAC value for aquatic invertebrates and primary producers. Step 3.

Scenario	PEC_{sw} (FOCUS Step 3)*		TER based on ETO-RAC of 1 µg a.s./L (trigger is 1)	TER based on ERO-RAC of 3.1 µg a.s./L (trigger is 1)
	Scenario	µg/L		
Spring cereals, 1 x 750 g a.s./ha	D1 Ditch	4.80	**0.21**	**0.65**
	D1 Stream	4.20	**0.24**	**0.74**
	D3 Ditch	4.76	**0.21**	**0.65**
	D4 Pond	0.164	6.10	18.9

	D4 Stream	4.09	**0.24**	**0.76**
	D5 Pond	0.164	6.10	18.9
	D5 Stream	4.42	**0.23**	**0.70**
	R4 Stream	3.14	**0.32**	**0.99**
Spring cereals, 2 x 750 g a.s./ha	D1 Ditch	5.93	**0.17**	N.A.
	D1 Stream	3.63	**0.28**	N.A.
	D3 Ditch	4.16	**0.24**	N.A.
	D4 Pond	0.229	4.37	N.A.
	D4 Stream	3.55	**0.28**	N.A.
	D5 Pond	0.231	4.33	N.A.
	D5 Stream	3.83	**0.26**	N.A.
	R4 Stream	5.07	**0.20**	N.A.
Winter cereals, 1 x 750 g a.s./ha	D1 Ditch	4.76	**0.21**	**0.65**
	D1 Stream	3.70	**0.27**	**0.84**
	D2 Ditch	4.78	**0.21**	**0.65**
	D2 Stream	3.95	**0.25**	**0.78**
	D3 Ditch	4.74	**0.21**	**0.65**
	D4 Pond	0.164	6.10	18.9
	D4 Stream	3.51	**0.28**	**0.88**
	D5 Pond	0.164	6.10	18.9
	D5 Stream	3.79	**0.26**	**0.82**
	D6 Ditch	4.79	**0.21**	**0.65**
	R1 Pond	0.194	5.15	16.0
	R1 Stream	3.14	**0.32**	**0.99**
	R3 Stream	4.39	**0.23**	**0.71**
	R4 Stream	3.13	**0.32**	**0.99**
Winter cereals, 2 x 750 g a.s./ha	D1 Ditch	4.19	**0.24**	N.A.
	D1 Stream	3.54	**0.28**	N.A.
	D2 Ditch	4.18	**0.24**	N.A.
	D2Stream	3.42	**0.29**	N.A.
	D3 Ditch	4.15	**0.24**	N.A.
	D4 Pond	0.203	4.93	N.A.
	D4 Stream	3.13	**0.32**	N.A.
	D5 Pond	0.232	4.31	N.A.
	D5 Stream	3.62	**0.28**	N.A.
	D6 Ditch	4.32	**0.23**	N.A.
	R1 Pond	0.235	4.26	N.A.
	R1 Stream	2.82	**0.35**	N.A.
	R3 Stream	3.82	**0.26**	N.A.
	R4 Stream	2.71	**0.37**	N.A.
Tomatoes, 1 x 1000 g a.s./ha	D6 Ditch	6.29	**0.16**	**0.49**
	R2 Stream	5.61	**0.18**	**0.55**
	R3 Stream	5.90	**0.17**	**0.53**

	R4 Stream	5.42	**0.18**	**0.57**

From table B.9.4-19 it appears that in the case of the ETO-RAC as well as the ERO-RAC, for all scenarios for the different uses, except the pond scenarios, the TER is below the trigger value of 1 . Hence, a further refinement is necessary.

Refinement (FOCUS Step 4 values), based on ETO-RAC

As a refinement the notfier has submitted FOCUS step 4 values. Risk mitigation measures are proposed (Run-off mitigation in combination with spray-drift buffers). In table B.9.4-20 - 22 the FOCUS step 4 PECsw values are presented for the different uses and scenarios. The ETO-RAC is 1.0 µg a.s./L . FOCUS Step 4 values above this value are in bold.

Table B.9.4-20: Comparison of exposure scenarios following application of chlorothalonil to spring cereals at FOCUS Step 4 to the ETO-RAC of 1.0 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Spring cereals 1 x 750 g a.s./ha BBCH 30	D1	Ditch	0.690	-	-
	D1	Stream	0.814	-	-
	D3	Ditch	0.684	-	-
	D4	Pond	-	-	-
	D4	Stream	0.793	-	-
	D5	Pond	-	-	-
	D5	Stream	0.857	-	-
	R4	Stream	0.608	-	-
Spring cereals 2 x 750 g a.s./ha BBCH 30	D1	Ditch	0.786	-	-
	D1	Stream	0.667	-	-
	D3	Ditch	0.561	-	-
	D4	Pond	-	-	-
	D4	Stream	0.651	-	-
	D5	Pond	-	-	-
	D5	Stream	0.702	-	-
	R4	Stream	**5.07**	**2.28**	**1.19**

PEC values in bold are greater than the ETO-RAC of 1.0 µg/L

Table B.9.4-21: Comparison of exposure scenarios following application of chlorothalonil to winter cereals at FOCUS Step 4 to the ETO-RAC of 1.0 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{SW} [µg/L]		
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Winter cereals 1 x 750 g a.s./ha BBCH 30	D1	Ditch	0.684	-	-
	D1	Stream	0.717	-	-
	D2	Ditch	0.687	-	-
	D2	Stream	0.766	-	-
	D3	Ditch	0.681	-	-
	D4	Pond	-	-	-
	D4	Stream	0.679	-	-
	D5	Pond	-	-	-
	D5	Stream	0.734	-	-
	D6	Ditch	0.688	-	-
	R1	Pond	-	-	-
	R1	Stream	**2.82**	**1.28**	0.667
	R3	Stream	0.851	-	-
	R4	Stream	0.606	-	-
Winter cereals 2 x 750 g a.s./ha BBCH 30	D1	Ditch	0.565	-	-
	D1	Stream	0.649	-	-
	D2	Ditch	0.563	-	-
	D2	Stream	0.627	-	-
	D3	Ditch	0.559	-	-
	D4	Pond	-	-	-
	D4	Stream	0.575	-	-
	D5	Pond	-	-	-
	D5	Stream	0.664	-	-
	D6	Ditch	0.582	-	-
	R1	Pond	-	-	-
	R1	Stream	**2.82**	**1.28**	0.667
	R3	Stream	**3.36**	**1.53**	0.804
	R4	Stream	**1.63**	0.736	-

PEC values in bold are greater than the ETO-RAC of 1.0 µg/L

Table B.9.4-22: Comparison of exposure scenarios following application of chlorothalonil to tomatoes at FOCUS Step 4 to the ETO-RAC of 1.0 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{SW} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Tomatoes 1 x 1000 g a.s./ha BBCH 51	D6	Ditch	0.904	-	-
	R2	Stream	**1.09**	**1.09**	0.565
	R3	Stream	**1.17**	**1.14**	0.594
	R4	Stream	**5.42**	**2.46**	**1.29**

PEC values in bold are greater than the ETO-RAC of 1.0 µg/L

The resulting comparison of FOCUS Step 3 and 4 PEC_{SW} values to the ETO-RAC of 1.0 µg/L indicate that the risk to aquatic invertebrates and primary producers is acceptable following the use of A14111B according to the proposed use pattern with consideration given to appropriate mitigation requirements as presented in Table B.9.4-23.

Table B.9.4-23: Mitigation requirements for acute risk to aquatic invertebrates and primary producers, based on the ETO-RAC of 1.0 µg a.s./L

Crop	Appl. Rate (g/ha)	No. of appl.	Scenario									
			D1	D2	D3	D4	D5	D6	R1	R2	R3	R4
Spring cereals	750	1	10 m SD		10 m SD	10 m SD	10 m SD					10 m SD
		2	10 m SD		10 m SD	10 m SD	10 m SD					>**M**
Winter cereals	750	1	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD with 60% RO		10 m SD	10 m SD
		2	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD	20 m SD with 80% RO		20 m SD with 80% RO	10 m SD with 60% RO
Tomatoes	1000	1						10 m SD		20 m SD with 80% RO	20 m SD with 80% RO	>**M**

A grey field means that the scenario is not relevant for this crop group
RO = run-off mitigation; SD = spray drift buffer
>M = mitigation greater than 80% run-off + 20 m spray buffer is required

The only scenario which does not achieve acceptable mitigation is R4 stream in spring cereals and tomatoes. For this scenario further risk reduction measures are necessary.

Refinement (FOCUS Step 4 values), based on ERO-RAC

As a refinement the notfier has submitted FOCUS step 4 values. Risk mitigation measures are proposed (Run-off mitigation in combination with spray-drift buffers). In table B.9.4-24 - 26 the FOCUS step 4 PECsw values are presented for the different uses and scenarios. The ERO-RAC is 3.1 µg a.s./L . FOCUS Step 4 values above this value are in bold. However, it should be pointed out that the practical use of an ERO-RAC will be difficult to justify (see EFSA 2013, section 5.5 point 2).

Table B.9.4-24: Comparison of exposure scenarios following application of chlorothalonil to spring cereals at FOCUS Step 4 to the ERO-RAC of 3.1 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Spring cereals 1 x 750 g a.s./ha BBCH 30	D1	Ditch	0.690	-	-
	D1	Stream	0.814	-	-
	D3	Ditch	0.684	-	-
	D4	Pond	-	-	-
	D4	Stream	0.793	-	-
	D5	Pond	-	-	-
	D5	Stream	0.857	-	-
	R4	Stream	0.608	-	-
Spring cereals 2 x 750 g a.s./ha BBCH 30	D1	Ditch	0.786	-	-
	D1	Stream	0.667	-	-
	D3	Ditch	0.561	-	-
	D4	Pond	-	-	-
	D4	Stream	0.651	-	-
	D5	Pond	-	-	-
	D5	Stream	0.702	-	-
	R4	Stream	**5.07**	2.28	1.19

PEC values in bold are greater than the ERO-RAC of 3.1 µg/L

Table B.9.4-25: Comparison of exposure scenarios following application of chlorothalonil to winter cereals at FOCUS Step 4 to the ERO-RAC of 3.1 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{sw} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Winter cereals 1 x 750 g a.s./ha BBCH 30	D1	Ditch	0.684	-	-
	D1	Stream	0.717	-	-
	D2	Ditch	0.687	-	-
	D2	Stream	0.766	-	-
	D3	Ditch	0.681	-	-
	D4	Pond	-	-	-
	D4	Stream	0.679	-	-
	D5	Pond	-	-	-
	D5	Stream	0.734	-	-
	D6	Ditch	0.688	-	-
	R1	Pond	-	-	-
	R1	Stream	2.82	1.28	0.667
	R3	Stream	0.851	-	-
	R4	Stream	0.606	-	-

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{SW} [µg/L]		
Winter cereals 2 x 750 g a.s./ha BBCH 30	D1	Ditch	0.565	-	-
	D1	Stream	0.649	-	-
	D2	Ditch	0.563	-	-
	D2	Stream	0.627	-	-
	D3	Ditch	0.559	-	-
	D4	Pond	-	-	-
	D4	Stream	0.575	-	-
	D5	Pond	-	-	-
	D5	Stream	0.664	-	-
	D6	Ditch	0.582	-	-
	R1	Pond	-	-	-
	R1	Stream	2.82	1.28	0.667
	R3	Stream	**3.36**	1.53	0.804
	R4	Stream	1.63	0.736	-

PEC values in bold are greater than the ERO-RAC of 3.1 µg/L

Table B.9.4-26: Comparison of exposure scenarios following application of chlorothalonil to tomatoes at FOCUS Step 4 to the ERO-RAC of 3.1 µg a.s./L

Crop (Use pattern)	Scenario	Water body	FOCUS Step 4 PEC_{SW} [µg/L]		
Run-off mitigation			**-**	**60%**	**80%**
Spray-drift buffer			**10 m**	**10 m**	**20 m**
Tomatoes 1 x 1000 g a.s./ha BBCH 51	D6	Ditch	0.904	-	-
	R2	Stream	1.09	1.09	0.565
	R3	Stream	1.17	1.14	0.594
	R4	Stream	**5.42**	2.46	1.29

PEC values in bold are greater than the ETO-RAC of 3.1 µg/L

The resulting comparison of FOCUS Step 3 and 4 PEC_{SW} values to the ETO-RAC of 3.1 µg/L indicate that the risk to aquatic invertebrates and primary producers is acceptable following the use of A14111B according to the proposed use pattern with consideration given to appropriate mitigation requirements as presented in Table B.9.4-27.

Table B.9.4-27: Mitigation requirements for acute risk to aquatic invertebrates and primary producers, based on ERO-RAC of 3.1 µg a.s./L

Crop	Appl. Rate (g/ha)	No. of appl.	Scenario									
			D1	D2	D3	D4	D5	D6	R1	R2	R3	R4
Spring cereals	750	1	10 m SD		10 m SD	10 m SD	10 m SD					10 m SD
		2	N.A.		N.A.	N.A.	N.A.					N.A.
Winter cereals	750	1	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD	10 m SD		10 m SD	10 m SD
		2	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.
Tomatoes	1000	1						10 m SD		10 m SD	10 m SD	10 m SD with 60% RO

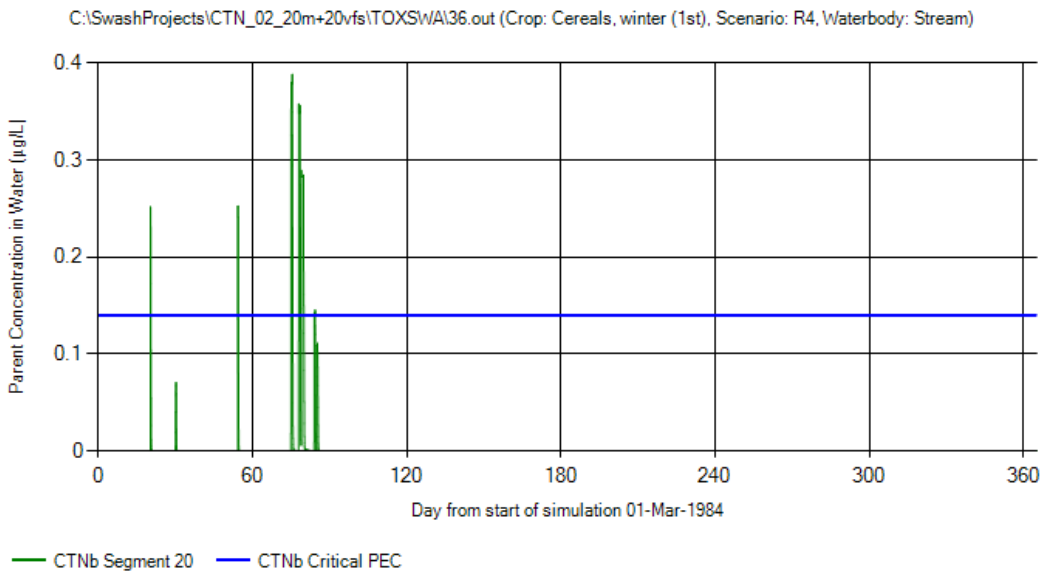
A grey field means that the scenario is not relevant for this crop group
RO = run-off mitigation; SD = spray drift buffer
>M = mitigation greater than 80% run-off + 20 m spray buffer is required

Refinement of the risk to aquatic invertebrates for the R4 scenario

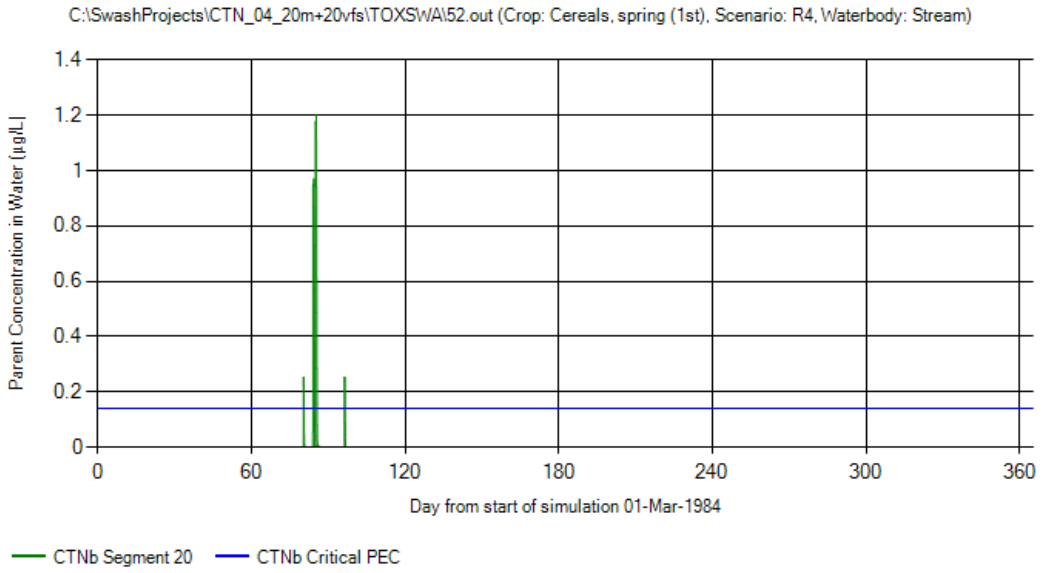
The notifier does not agree with the approach of the RMS with regards to the endpoints derived from both mesocosm studies. In case of the Tattersfield (2002) study the RMS applied an AF 3 instead of 2 (EFSA 2013) with the ETO-RAC and no ERO-RAC was derived due to the uncertainty about the worst-casedness of the exposure in the study.

The notifier is of opinion that "*dissipation is certainly realistic as it is a field study, furthermore dissipation in the models, such as the stream scenarios is not driven by the DT50 as much as the water flow and so the exposure scenarios from the mesocosm study with dissipation from peak concentrations with a DT50 of approximately 8 hours is indeed worst case compared to the stream run-off scenarios , as can be seen from the profiles in the EPAT modelling (see Chlorothalonil EPAT files Syngneta. Ref R044686_11488)*".

R4 Stream, Winter cereals 2 * 750 g/ha



R4 Stream, Spring cereals 2 * 750 g/ha



During the Pesticides Peer review Meeting 165 (18-22 September 2017) the following was concluded regarding the comparison between the measured concentrations in the cosms and the FOCUS exposure profiles:

A non-exhaustive comparison between the measured concentrations in the cosm and some FOCUS profiles was presented at the meeting by the RMS as provided by the Applicant. This information was not available in the RAR. Only some scenarios were reported and the RMS clarified that they were FOCUS step 4. Generally, it was not possible to achieve a conclusion on whether the dissipation in the mesocosm was realistic with all exposure profiles of the representative uses under evaluation. A data gap was considered needed for a full comparison with FOCUS profiles. Pending on the outcome of this data gap, uncertainty should be acknowledged on the NOEC used for deriving the ETO-RAC i.e. currently the NOEC is expressed as nominal concentration; additional work is needed to check if the exposure in the cosm is realistic with the field exposure of the representative uses.

A data gap was identified for the applicants to provide a complete assessment of the exposure profile in the cosm and their comparison with the FOCUS profiles.

Hence, no final conclusion can be drawn regarding the risk assessment for aquatic invertebrates based on the outcome of the submitted mesocosm studies. A complete assessment of the exposure profile in the mesocosm and their comparison with the FOCUS profiles has to be provided.

B.9.4.3.3 Metabolites

The notifier has submitted the following statement regarding metabolites of chlorothalonil:
The occurrence of potentially ecotoxicologically relevant metabolites has been considered and are discussed in M-CP Section 9. Soil organisms could potentially be exposed to soil metabolites, as could aquatic organisms. In addition, the EFSA Aquatic Guidance states that the sediment/water metabolism and the aerobic mineralistation in surface water studies should be considered to identify potentially ecologically relevant metabolites. A large amount of data are available to assess the risk from the metabolites, including ecotoxicological testing, fungicidal activity, as well as glutathione reactivity (the basis of the biological activity of chlorothalonil). Environmental metabolism generally involves the replacement of one or more of the Cl or CN groups. Although highly complex there are clear structural similarities between many of the metabolites of chlorothalonil. This was recognised in the EU Assessment Report and agreed that for risk assessment purposes R182281, R611965 and R417888 represented the major structural groupings. Accordingly, as is the case for toxicological purposes, it is considered that R417888 and R611965 cover the other sulphonic and carboxylic acid metabolites for ecotoxicology. The water sediment study identified metabolites not found in the soil metabolism, R613841, R613842 and R613801, these have also been tested for toxicity to aquatic organisms. All the relevant soil and water metabolites that have been tested are of much lower toxicity than the parent to aquatic organisms. Soil metabolites have been shown to be of similarly low toxicity or lower toxicity than the parent to soil organisms. None of the potential soil metabolites tested have shown any biological activity in fungicidal testing (R182281, R417888, R611965, R613636, R611968,, SYN507900, R419492, R471811, R418503, SYN548008, SYN548580 and SYN548581) or glutathione recativity (R182281, R417888, R611965, R613636, R611968, SYN548580, SYN548581, SYN548008, R419492, R471811), which is the biological basis for chlorothalonil's activity and so would not be expected to show any significant non-target toxicity (see documents MCA Section 8 and N4).

The RMS agrees with the notifier, all metabolies are covered. In addition, the groundwater metabolites (M3 (SYN548008), M11 (SYN548581), M2, M7 and M10) from the lysimeter study are also covered by the risk assessment below. The surface water metabolites which have been tested in ecotoxicology studies are presented in the table below.

Table B.9.4-28 Ecotoxicologically potentially relevant metabolites of chlorothalonil

Compartment	Ecotoxicologically relevant metabolites
Surface water	R182281 (=SDS 3701), R611965 (=SDS 46851), R417888, R613841, R613842, R613801

The risk to aquatic organisms from the chlorothalonil metabolites tested is presented in Table B.9.4-29. The metabolites represent the major metabolites, in terms of PECs within surface water and are representatives of structural groupings.

Table B.9.4-29: Risk to aquatic organisms from chlorothalonil metabolites

Test species	Metabolite	End-point	Value (mg/L)	RAC (µg/L)	Max FOCUS Step 2 PEC (µg/L)	TER (trigger is 1)
Fish						
Oncorhynchus mykiss	R417888	96-h acute LC_{50}	> 100	>1000	29.1	>34.4
	R182281 (SDS-3701)		9.1	91	46.6	1.95
	R611965 (SDS-46851)		> 120	>1200	20.5	>58.5
	R613841 (SDS-67042)		> 0.83	>8.3	21.6	**>0.38**
	R613842 (SDS-67042 sulphoxide)		> 0.99	>9.9	7.30	>1.36
	R613636 (SDS-19221)		18	180	33.9	5.31
Aquatic invertebrates						
Daphnia magna	R417888	48-h acute EC_{50}	> 110	>1100	29.1	>37.8
Mysidopsis bahia	R182281 (SDS-3701)		19	190	46.6	4.08
Daphnia magna	R611965 (SDS-46851)		> 100	>1000	20.5	>48.8
	R613841 (SDS-67042)		> 0.94	>9.4	21.6	**>0.44**
	R613842 (SDS-67042 sulphoxide)		>0.89	>8.9	7.30	>1.22
	R613636 (SDS-19221)		12.4	124	33.9	3.66
	R613801		0.56	5.6	9.66	**0.58**
Algae						
Pseudokirchneriella subcapitata	R182281 (SDS-3701)	72-h E_bC_{50}	14.2	1420	29.1	30.5
	R417888	72 h E_rC_{50}	>100	>10000	46.6	>344
	R611965 (SDS-46851)		55	5500	20.5	268
Pseudokirchneriella subcapitata	R613841 (SDS-67042)		>0.041	4.1	21.6	**>0.19**
Pseudokirchneriella subcapitata	R613842 sulfoxide (SDS-67042)		>0.88	>88	7.30	>12.1
	R613636 (SDS-19221)		12	1200	33.9	35.4
	R613801		0.38	38	9.66	3.93

Looking at the TER values of table B.9.4-29 it appears that all TER values are above the trigger value, except the TER of the metabolite R613841 with respect to fish, aquatic invertebrates and algae and the metabolite R613801 with respect to aquatic invertebrates. The notifier did not submit FOCUS Step 3 and 4 PEC_{sw} but argued "*R613801 is a photolytic metabolite and can only be formed from parent in the water phase with a maximum rate of formation of 20.5%. The maximum PEC at step 4 after refinements for the parent is 1.33 µg/L, occurring in stream scenarios with no potential for any build up of concentration from multiple entry events. At this concentration of parent the maximum concentration of it's aqueous photolysis metabolite formed at 20.5 % and corrected for molecular weight can only be 0.24 µg/L, well below the RAC of 5.6 µg/L*". The metabolite R613841 was not addressed. However, this concerns a sediment metabolite. The RMS is of opinion that based on the high margin of safety regarding the risk assessment of the parent (acceptable risk at FOCUS Step 2) and considering risk mitigation measures are required for the active substance (see above), the risk is considered acceptable. It can be concluded that the toxicity of the metabolites is lower than the toxicity of the active substance for all taxonomic groups.

B.9.4.4 Assessment of bioconcentration potential

As the logPow of chlorothalonil is < 3 (0.8), the potential for bioconcentration is considered to be low.

B.9.4.5 Conclusions

- The acute risk to fish is acceptable for all FOCUS scenarios based on FOCUS Step 4 values for all uses applied for.
- The chronic risk to fish is not acceptable. All FOCUS Step 4 values are above the chronic RAC for fish (1.4/10 = 0.14 µg a.s./L), except for the pond scenarios.
- The acute risk to amphibians is not acceptable. All FOCUS Step 4 values are above the chronic RAC for fish (1.4/10 = 0.14 µg a.s./L), except for the pond scenarios.
- The long term risk to amphibians cannot be finalized (see also B.9.4.6, below). A data gap is identified.
- The acute and long term risk to aquatic invertebrates and the risk to primary producers cannot be finalized. A data gap is identified.
- The risk from the metabolites is acceptable for all uses applied for.

B.9.4.6 Endocrine disruption

The AMA shows that chlorothalonil affects the thyroid in *Xenopus* at low exposure levels. No thyroid effects were seen in the mammalian toxicology section, however, amphibians are more sensitive to thyroid perturbation than mammals. A LAGDA assay has been requested in order to investigate the relevance of these effects on the risk assessment for amphibians (see section 9.4.2, above), however, the notifier has indicated that this assay has not been performed, and is not planned, as no test order

has been issued by the US EPA. The results of the study Zhang, et. al., indicate that the metabolite SDS-3701 interacts with the TRβ. Considering this, it is possible that the metabolite is the cause of the thyroid effects seen in *Xenopus*. The metabolism of chlorothalonil to SDS-3701 appears to be highly variable in different species, and may occur at a higher level in *Xenopus* than in the tested mammalian species, explaining the lack of significant thyroid indications in the mammalian toxicology dataset, particularly the male and female pubertal rat thyroid development assays with chlorothalonil. The rest of the mammalian data set is highly variable where thyroid effects are concerned. In most instances, the thyroid is not considered. In several cases, including long term studies with mice and dog, thyroid weight is increased, and there were some histopathological changes and increases in parathyroid weight in rats, however, these effects were inconsistent between males and females, and across species. However, the ecotoxicological data set seems to show clear evidence of some effect on thyroid, as there were effects on thyroid in the AMA, and Zhang et. al. reported interaction of the metabolite SDS-3701 with the TRβ. The RMS cannot conclude by what pathway these effects occur, nor whether the effects are directly attributable to chlorothalonil, or to the metabolite SDS-3701, however, it can be concluded that exposure to chlorothalonil results in changes in *Xenopus* thyroid at relatively low doses.

The FSTRA shows that chlorothalonil affects fecundity in fathead minnow at low exposure levels. Decreased egg production was also seen in the FFLC. Both of these effects were seen in the absence of overt toxicity, however, neither is sufficient to determine whether an endrogen pathway interaction is responsible for these effects. Since the endpoint used in the above risk assessment is based on decreased egg production in the FFLC test, the risk assessment can be concluded to be protective of population level reproductive effects in fish.

B.9.5 Effects on arthropods

B.9.5.1 Effects on bees

Table B.9.5.1-01: Summary of available toxicity data for bees

Organism	Test item	Test type	EU endpoint[a]	Endpoint used in the risk assessment	Reference
Apis mellifera	Chlorothalonil	48h oral	LD_{50} >40 µg/bee	LD_{50} >63 µg/bee	***Cole (1992) VCM 7/911157***
		48h contact	LD_{50} >63 µg/bee	LD_{50} >101 µg/bee	***Thompson (2000) R44686/0186***
		Adult Chronic		10 d NOEC = 188 mg a.s./kg diet; ca. 6.5 µg a.s./bee/day LD_{50} = 53.9 µg a.s./bee/day.	***Kleebaum (2014) A7867A_11245***
		Larval development	-	7 d NOEC = 91 mg a.s./kg diet (14.5 µg total a.s./larva = 3.6 µg a.s./larva/day	***Kleebaum (2014) A7867A_11246***
	A14111B	48h oral	-	LD_{50} = >917 µg/bee (> 317 µg chlorothalonil/bee)	***Bocksch (2004) ICI5504/2259***
		48h contact	-	LD_{50} >1531 µg/bee (> 523 µg chlorothalonil/bee)	
		Adult	-	NOEC = 606 mg/kg food;	***Ruhland (2014)***

		Chronic		NOED = 29.1 µg/bee/day LD_{50} = 171 µg/bee/day	
		Larval development	-	8 d NOEC = 198 mg/kg diet NOED =31.3 µg total prod/larva = 7.8 µg prod/larva/day	***Kleebaum (2015) A14111B/11218***
Bombus terrestris	Chlorothalonil	96 h oral		LD_{50} >94 µg/bee	***Fauser-Misslin (2015) R44686/11179***
		96 h contact		LD_{50} >100 µg/bee	

B.9.5.1.1 Acute toxicity to bees

Acute oral toxicity to bees

A summary of a study conducted using the representative formulation is presented below.

Report:	K-CP 10.3.1.1.1/01, Bocksch S., (2004). Azoxystrobin / Chlorothalonil (ZA5504 / RO44686) 80 / 400 SC (A14111B): Acute oral and contact toxicity of a 480 g/L SC formulated mixture to the honeybee, *Apis mellifera* L. in the laboratory. Report Number 20031441/S1-BLEU, GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. (Syngenta file No. ICI5504/2259)
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. The 48-hour oral LD_{50} for A14111B was > 917 µg formulation/bee (corresponding to > 317 µg chlorothalonil/bee) and the 48 hour contact LD_{50} was >1531 µg formulation/bee (corresponding to > 523 µg chlorothalonil/bee).

Guidelines

OECD Guideline 213 Honeybees, Acute Oral Toxicity Test (1998); OECD Guideline 214 Honeybees, Acute Contact Toxicity Test (1998).

GLP: Yes.

Executive Summary

Worker honey bees (*Apis mellifera*) were exposed to A14111B by contact and oral exposure. The dose for the contact test was 1513 µg formulation/bee. In the oral test, the target dose was the same as in the contact test, but consumption data indicated a dose of 917 µg/ bee. The 48-hour oral LD_{50} for A14111B was > 917 µg A1411B /bee (corresponding to > 317 µg chlorothalonil/bee) and the 48 hour contact LD_{50} was > 1531 µg A1411B /bee (corresponding to > 523 chlorothalonil/bee).

Materials

Test Material: A14111B

Description: Cream opaque liquid

Lot/Batch #: J7518/024

Purity: 80 g/L azoxystrobin (6.6 % (w/w)) and 419 g/L chlorothalonil (34.6 %

	(w/w)) (analyzed)
Stability of test compound:	Assumed stable pending re-analysis in September 2005
Density:	1.21 g/mL
Test concentrations:	Contact test: 1513 µg formulation/bee; Oral test: the target dose was the same as in the contact test, but consumption data indicated a dose of 917 µg/ bee.
Vehicle and control:	Oral toxicity test: 50% aqueous sucrose solution Contact toxicity test: tap water
Toxic reference:	Dimethoate
Test organisms	
Species:	*Apis mellifera*
Source:	Culture descending from a breeding line of a beekeeper in Ayora, Spain
Food:	50% aqueous sucrose solution *ad libitum*
Environmental test conditions	During the experimental phase the test animals were kept in darkness
Temperature:	24 - 25 °C
Humidity:	56 – 84 % relative humidity

Study Design and Methods

Experimental dates: 27 January – 5 February 2004.

Worker honey bees (*Apis mellifera*) were exposed to A14111B by contact and oral exposure. At each concentration and treatment, respectively, five replicate groups of 10 bees were tested. The dose for the contact test was 1513 µg formulation/bee. In the oral test, the target dose was the same as in the contact test, but consumption data indicated a dose of 917 µg/ bee. A toxic standard (dimethoate) was included.

For the oral toxicity test, the test substance was added to tap water to make a stock solution. The final dose was prepared by mixing an appropriate amount of the stock solution with an appropriate amount of 50 % w/v aqueous sucrose solution, such that 20 µL contained the required amount of test item per bee, even though 25 µL was provided. Before bees were permitted to feed, they were starved for 2 hours. A quantity of 250 µL of test item and reference item solution was offered for 6 hours to each cage of 10 bees to ensure sufficient consumption of test or reference item. Bees within a cage share food by tropholaxis and therefore are assumed to have received a similar dose. The amount of test solution consumed by each replicate (consisting of 10 bees) was determined by weighing the feeders before and after feeding. After the test solutions were consumed, the bees were supplied *ad libitum* with untreated 50% aqueous sucrose solution.

For the contact toxicity test, the test substance was added to tap water. After the bees had been anaesthetised with carbon dioxide they were treated individually by topical application with a microapplicator. 4 µL of test item and tap water, and 2µL of reference item solution were applied to

the thorax of each bee, respectively. After application the bees were returned to the test cages and fed with a 50% aqueous sucrose solution *ad libitum*.

The number of dead bees in the individual test cages was recorded after 4 h, 24 h and 48 h in the oral and contact test. In case of symptoms of poisoning the behavioural differences between the bees of the control group and those of the test item treatment were noted at each observation interval.

Results and Discussion

No behavioural abnormalities or mortalities of the bees that could be attributed to the exposure to the test item were observed during the test. Consequently, the 24 and 48-hour oral LD_{50} values based on mean actual uptake were both > 917 μg formulation/bee and the 24 and 48-hour contact LD_{50} values were both > 1513 μg formulation/bee.

Control mortality of 2 and 6% were observed in the oral and contact toxicity tests, respectively, within the 48 hours observation period.

The 24 hour contact and oral LD_{50} values for the reference item were 0.22 and 0.16 µg dimethoate/bee, respectively. Consequently, validity criteria for both control and reference item mortality were met and the test was deemed valid.

Conclusion

The 48-hour oral LD_{50} for A14111B was > 917 µg formulation/bee (corresponding to > 317 µg chlorothalonil/bee) and the 48 hour contact LD_{50} was >1531 µg formulation/bee (corresponding to > 523 chlorothalonil/bee).

(Bocksch S. 2004)

Acute contact toxicity to bees

Please refer to the summary presented above in CP 10.3.1.1.1. Studies have been conducted with the current representative formulation A14111B under current guidelines which will be used in the risk assessment.

B.9.5.1.2 Chronic toxicity to bees

A summary of a study conducted using the representative formulation is presented below.

Report:	K-CP 10.3.1.2/01, Ruhland K (2014). Azoxystrobin/Chlorothalonil SC (A14111B) - Chronic Toxicity to the Honeybee *Apis mellifera* L. in a 10 Day Continuous Laboratory Feeding Study. Report Number 14 10 48 058 B, Biochem agrar, Germany. (Syngenta file No. A14111B_11202)
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. The LC_{50} was 5.454 g A14111B/kg food (corresponding to 1.7 g chlorothalonil/kg food) and the NOEC was 0.606 g A14111B/kg food (corresponding to 0.19 g chlorothalonil/kg food). The LD_{50} was 171.0 µg A14111B/bee/day (corresponding to 54 µg chlorothalonil/bee/day or a cumulative dose of 540 µg chlorothalonil/bee) and the NOED was 29.1 µg A14111B/bee/day (corresponding to 9.2 µg

	chlorothalonil/bee/day or a cumulative dose of 92 µg chlorothalonil/bee) .

Guidelines

Decourtye A, *et al.* Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*, 2005

Suchail S *et al.*: Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera,* 2001

AFPP Method No. 230: Evaluation of effects of plant protection products on *Apis mellifera* L. (French Association for Plant Protection: Guideline for chronic toxicity testing, 2012)

EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees). EFSA Journal 11(7): 3295, 266 pp., 2013

AG-Bienenschutz, International ring test protocol: Adult honeybee (*Apis mellifera* L.), Chronic toxicity test (10 day feeding test in the laboratory) (Method validation), 2014

GLP: Yes

Executive Summary

The effects of A14111B were assessed on young adult honey bees, *Apis mellifera*, in a 10 day chronic feeding test under laboratory conditions.

The LC_{50} was calculated to be 5.454 g A14111B/kg food (corresponding to 1.7 g chlorothalonil/kg food) and the NOEC was determined to be 0.606 g A14111B/kg food (corresponding to 0.19 g chlorothalonil/kg food).

The LD_{50} was calculated to be 171.0 µg A14111B/bee/day (corresponding to 54 µg chlorothalonil/bee/day or a cumulative dose of 540 µg chlorothalonil/bee) and the NOED was determined to be 29.1 µg A14111B/bee/day (corresponding to 9.2 µg chlorothalonil/bee/day or a cumulative dose of 92 µg chlorothalonil/bee) .

Test Material	A14111B Azoxystrobin/chlorothalonil SC (080/400)
Lot/Batch #:	GRA1A063B/1
Actual content of active ingredients:	Chlorothalonil: 31.7 % w/w corresponding to 384 g/L Azoxystrobin: 6.17 % w/w corresponding to 74.7 g/L
Description:	Yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	31 December 2014
Density:	1210 kg/m^3
Treatments	
Test rates:	Nominal: 23.6, 47.2, 94.4, 188.8 and 377.5 µg A14111B/bee/day (0.606, 1.212, 2.424, 4.847 and 9.695 g A14111B/kg food)
Control:	50 % (w/v) aqueous sucrose solution
Toxic standard:	Dimethoate 400 EC (nominally 400.0 g/L (400.9 g/L, analysed)); tested at nominal rates of 5.9, 9.8, 16.4 and 27.3 ng dimethoate/bee/day (0.152, 0.253, 0.421 and 0.702 mg dimethoate/kg food)

Administration:	Ingestion in aqueous sucrose solution
Test organisms	
Species:	*Apis mellifera* L. (Hymenoptera, Apoidae) subspecies *carnica*
Source:	Healthy young female worker bees (1 to 4 days old) derived from a colony obtained from Bienenfarm Kern GmbH, Am Rehbacher Anger 10, 04249 Leipzig, Germany
Food:	50 % w/v aqueous sucrose solution
Test design	
Test cage description:	Aluminium cages (20 x 15 x 10 cm) with holes in the lateral walls for sufficient air supply, and two glass plates (in the front and back) for observation
Replication:	3
No. of bees/arena :	20
Duration of test:	10 days
Environmental test conditions	
Temperature:	32.7 – 33.1 °C
Humidity:	58.0 – 62.0 % (RH)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 29 July 2014 to 08 August 2014

Four days prior to test initiation, brood combs containing capped cells which were expected to hatch on the same day were transferred into a climatically controlled chamber from the honey bee colony. Brood combs were taken from different colonies. One day prior to test start the newly-hatched bees were transferred from combs to the test cages and kept under test conditions.

Feeding solutions were placed in plastic syringes and offered to the bees in each unit *ad libitum*. Bees in one replicate shared the feeding solution and thus received similar doses. Feeding solutions were replaced daily and the amount of feeding solution consumed was determined by weighing the syringe before and after feeding.

Mortality was recorded every 24 h after the start of feeding with the treated diet for 10 days.

The LC_{50} and LD_{50} values with 95 % confidence intervals of the test item group were calculated by means of Logit analysis using linear maximum likelihood regression. Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a difference between the mortality data of the test item and control groups and determine the NOEC and NOED. Statistical calculations were made using the statistical software ToxRat professional, Version 2.10.06 (2010) (ToxRat Solutions GmbH).

Results and Discussion

Table 10.3.1.2-1: Accumulated mean uptake of A14111B

Dose (g A14111B/kg food)	**Accumulated mean uptake of test item (µg A14111B/bee/day)**									
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**
0.606	17.4	42.0	67.3	96.6	124.	156.	189.	223.	256.	290.8

						5	5	6	1	8	
1.212		50.3	104.3	156.0	207.4	266.2	325.0	384.6	442.9	507.3	568.5
2.424		89.6	168.7	257.2	402.7	477.2	589.7	703.7	794.3	883.8	975.2
4.847		149.4	285.8	410.1	553.4	680.6	875.6	1025.2	1136.5	1234.7	1379.1
9.695		177.7	426.7	652.4	931.5	1201.8	1421.2	2143.0	2329.6	2548.9	2871.0
Reference Item	**0.152 mg a.s./kg food**	6.1	12.0	18.4	25.9	32.4	38.7	44.7	50.3	56.5	62.0
	0.253 mg a.s./kg food	10.3	19.1	30.2	40.3	48.9	59.3	67.7	74.8	81.7	88.9
	0.421 mg a.s./kg food	12.3	28.8	44.2	56.6	66.9	77.5	85.5	95.7	104.1	112.2
	0.702 mg a.s./kg food	21.1	42.6	58.2	74.7	85.4	102.2	120.2	135.0	156.2	183.6

D = Day

Mortality data for the test material and control are summarised in the table below.

Table 10.3.1.2-2: Summary of chronic toxicity of A14111B to honey bees (*Apis mellifera* L.)

Dose (g A14111B/kg food)		**Mean cumulative mortality (%)**									
		D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**
Control		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.606		1.7	1.7	1.7	1.7	1.7	1.7	3.3	3.3	3.3	3.3
1.212		0.0	3.3	3.3	3.3	5.0	5.0	5.0	5.0	5.0	10.0*
2.424		0.0	0.0	1.7	3.3	5.0	5.0	5.0	6.7	6.7	10.0*
4.847		0.0	0.0	0.0	0.0	0.0	3.3	8.3	21.7	35.0	43.3*
9.695		0.0	1.7	15.0	18.3	25.0	35.0	40.0	43.3	63.3	78.3*
Reference Item	**0.152 mg a.s./kg food**	0.0	0.0	0.0	0.0	1.7	1.7	1.7	1.7	1.7	3.3
	0.253 mg a.s./kg food	0.0	0.0	0.0	0.0	0.0	1.7	1.7	1.7	3.3	5.0
	0.421 mg a.s./kg food	0.0	0.0	0.0	0.0	0.0	0.0	1.7	5.0	8.3	15.0*
	0.702 mg a.s./kg food	0.0	0.0	0.0	5.0	35.0	51.7	66.7	71.7	83.3	88.3*
LC_{50}		5.454 g A14111B/kg food									
NOEC		0.606 g A14111B/kg food									
LD_{50}		171.0 µg A14111B/bee/day or 1710 µg A14111B/bee									

NOED	29.1 µg A14111B/bee/day or 291 µg A14111B/bee

*Statistically significantly different compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction α = 0.05; one sided greater)

D = Day

Calculations were performed with non-rounded values

Validity criteria

The validity criterion for the test was met:

- ≤ 15 % mean mortality in the control after 10 days of exposure (0 % observed)

Conclusions

The effects of A14111B were assessed on young adult honey bees, *Apis mellifera*, in a 10 day chronic feeding test under laboratory conditions.

The LC_{50} was calculated to be 5.454 g A14111B/kg food (corresponding to 1.7 g chlorothalonil/kg food) and the NOEC was determined to be 0.606 g A14111B/kg food (corresponding to 0.19 g chlorothalonil/kg food).

The LD_{50} was calculated to be 171.0 µg A14111B/bee/day (corresponding to 54 µg chlorothalonil/bee/day or a cumulative dose of 540 µg chlorothalonil/bee) and the NOED was determined to be 29.1 µg A14111B/bee/day (corresponding to 9.2 µg chlorothalonil/bee/day or a cumulative dose of 92 µg chlorothalonil/bee) .

(Ruhland S, 2014)

B.9.5.1.3 Effects on honey bee development and other honey bee life stages

Larval and brood development data for bees is a new data requirement under the **Annexes to Regulation 283/2013 and 284/2013**, applicable where there is a possibility that bees may be exposed. A summary of a study conducted using the representative formulation is presented below.

Report:	K-CP 10.3.1.3/01, Kleebaum K, (2015). Azoxystrobin/Chlorothalonil SC (A14111B) – Chronic toxicity to the honeybee larvae *Apis mellifera* L. under laboratory conditions (in vitro). Report Number 14 10 48 071 B, Biochem agrar, Germany. (Syngenta file No. A14111B_11218) .
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. The 8 day NOEC was 0.198 g A14111B/kg diet (corresponding to 0.066 g chlorothalonil/kg diet). The 8 day LD_{50} was 65.8 µg A14111B/larva (corresponding to 22 µg chlorothalonil/larva or 5.5 µg chlorothalonil/larva/day) and the NOED was 31.3 µg A14111B/larva or 7.8 µg A14111B/larva/day (corresponding to 10.4 µg chlorothalonil/larva or 2.6 µg chlorothalonil/larva/day).

Guidelines

OECD DRAFT Guidance Document for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (February 2014)
OECD 237 Guidelines for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, single exposure (2013)

GLP: Yes.

Executive Summary

The purpose of this study was to determine the chronic toxicity of A14111B to honeybee larvae *Apis mellifera* L. in an *in vitro* test after repeated oral application. The 8 day NOEC was determined to be 0.198 g A14111B/kg diet (corresponding to 0.066 g chlorothalonil/kg diet) . The 8 day LD_{50} was determined to be 65.8 µg A14111B/larva (corresponding to 22 µg chlorothalonil/larva or 5.5 µg a.s./larva/day) and the NOED was 31.3 µg A14111B/larva (corresponding to 10.4 µg chlorothalonil/larva or 2.6 µg a.s./larva/day).

Materials

Test Material	Azoxystrobin/Chlorothalonil SC A14111B
Lot/Batch #:	GRA4K222B
Actual content of active ingredients:	Azoxystrobin 6.74 % w/w corresponding to 82.4 g/L Chlorothalonil 33.3 % w/w corresponding to 407 g/L
Description:	Greyish liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of December 2017
Density:	1222 kg/m^3
Treatments	
Test rates:	Total µg A14111B/larva: 3.8, 10.9, 31.3, 89.4, 255.3 Total g A14111B/kg diet: 0.024, 0.069, 0.198, 0.565, 1.614
Control:	Untreated diet B for day 3; untreated diet C for days 4 - 6
Toxic standard:	Dimethoate tech. (BAS 152 I), purity 99.8 %
Application method:	Oral application using a sterile pipette
Test organisms	
Species:	Worker honey bee larvae *Apis mellifera* L. subspecies *iberica* G. (Insecta, Hymenoptera, Apoidea)
Age:	First instar (L1) during grafting
Source:	Purchased from Beekeeper Joaquin Cordero, Paseo de Colón No. 19, 41370 Cazalla (Sevilla), Spain
Food:	Aqueous sugar solution (50 % w/w each of royal jelly and sugar solution) Diet A: 12 % glucose, 12 % fructose, 2 % yeast Diet B: 15 % glucose, 15 % fructose, 3 % yeast Diet C: 18 % glucose, 18 % fructose, 4 % yeast

Test Design

Test cage description:	Crystal polysterene grafting cells placed in 48 well plates, wells were filled up to 1/3 with dental floss
Replication:	Control: 3 Test and reference item: 3
No. of larvae/replicate:	12
Environmental test conditions	
Temperature:	34.6 – 35.5 °C
Humidity:	91 – 99 % RH
Photoperiod:	Constant darkness
Duration of test:	Pre-grafting (*in vivo*): days -3 to 0 Grafting: day 1 Pre-exposure (*in vitro*): days 1 to 3 Application: days 3 to 6 Post exposure (*in vitro*): days 7 to 8

Study Design and Methods

Experimental dates: 1 February – 6 February 2015

The test/reference item was mixed into sterile filtered aqueous sugar solution. Several dilutions were prepared by adding further sugar solution. The royal jelly was added to each stock solution at a ratio of 1:1, based on (w/w), to reach the final test concentrations.

Honeybee larvae *Apis mellifera* L. were exposed to repeated oral application of 3.8, 10.9, 31.3, 89.4, 255.3 µg A14111B/larva (equivalent to 0.024, 0.069, 0.198, 0.565, 1.614 g A14111B/kg diet) in an *in vitro* test. One control group was included in the test. The larvae of the control treatment were fed with untreated artificial diet, which served as a vehicle for the test item and reference item. The reference item was applied once on Day 4.

On Day 1 the combs containing the larvae were transported from the hive to an acclimated laboratory room. Larvae were transferred from the combs to the crystal polystyrene grafting cells using a suitable grafting tool. During grafting the C-shaped larvae were placed on the surface of the artificial diet within the grafting cells. Cells were placed in 48 well plates filled up to 1/3 with a piece of dental roll. Each replicate unit consisted of 12 larvae, and there were 3 replicates per treatment and control. Each larva was fed daily between Day 3 and Day 6 using a sterile pipette.

The number of dead larvae was recorded on Days 4, 5, 6, 7 and 8. Any large amounts of unconsumed food or substantially undersized larvae were recorded on Days 7 and 8. After the last assessment (Day 8) the culture plates with all organisms were placed in a freezer.

All observations were made in comparison to the control larvae. For each concentration, the corrected mortality was calculated according to ABBOTT (1925) modified by SCHNEIDER-ORELLI (1947).

The LD_{10} and LD_{50} values were calculated by Weibull (maximum likelihood regression). The statistical significance of the mortality values and the NOEC was calculated suing Fisher's Exact Binomial Test with Bonferroni Correction ($P \leq 0.05$).

Results and Discussion

Mortality data and other observations (presence of unconsumed food, smaller body size of larva) for the test material and reference item are summarised in the table below.

Table 10.3.1.3-1: Summary of chronic toxicity of A14111B to honeybee larvae

Item applied	Dosage [µg A14111B/larva]	Concentration [g A14111B/kg diet]	Day 8		
			Mortality mean %		OO
			Absolute	Correct.	Mean %
Control	-	-	11.1	-	0.0
Test item	255.3	1.614	100.0*	100.0	-
	89.4	0.565	77.8*	75.0	50.0
	31.3	0.198	16.7	6.3	10.4
	10.9	0.069	16.7	6.3	10.0
	3.8	0.024	5.6	0.0	5.8
Reference item	6.2	0.039	63.9	59.3	13.3
Treatment	**Endpoints**		**Day 8**		
Test item doses	**LD_{50} [µg A14111B/larva][1] (95 %-CL)**		**65.8** (43.0 – 100.5)		
	NOED [µg A14111B/larva][2]		**31.3**		
Test item concentrations	**NOEC [g A14111B/kg/diet][2]**		**0.198**		

OO: Other observations

[1]: Lethal dose/concentration after 120 h exposure was calculated using Weibull analysis

[2]: Fisher's Exact Binomial test with Bonferroni Correction; α = 0.05

*: Statistically significant difference in pairwise comparison between treatment and untreated control (Fisher's Exact Binominal Test with Bonferroni; α = 0.05; one sided greater).

Validity Criteria

All of the validity criteria were met:

- Control cumulative mortality should be ≤ 15 % for larvae across all control replicates at day 8 (actual value 11.1 %)
- Reference item mortality should be ≥ 50 % for larvae across all reference replicates at day 8 (actual corrected value 59.3 %)

Conclusions

The purpose of this study was to determine the chronic toxicity of A14111B to honeybee larvae *Apis mellifera* L. in an *in vitro* test after repeated oral application. The 8 day NOEC was determined to be 0.198 g A14111B/kg diet (corresponding to 0.066 g chlorothalonil/kg diet). The 8 day LD_{50} was determined to be 65.8 µg A14111B/larva (corresponding to 22 µg chlorothalonil/larva or 5.5 µg a.s./larva/day) and the NOED was 31.3 µg A14111B/larva (corresponding to 10.4 µg chlorothalonil/larva or 2.6 µg a.s./larva/day).

(Kleebaum, 2015)

B.9.5.1.4 Sub-lethal effects

As the risk to bees is acceptable following use of A14111B according to the proposed use pattern, further tests are not necessary.

B.9.5.1.5 Cage and tunnel tests

As the risk to bees is acceptable following use of A14111B according to the proposed use pattern, further tests are not necessary.

B.9.5.1.6 Field tests with honeybees

As the risk to bees is acceptable following use of A14111B according to the proposed use pattern, further tests are not necessary.

B.9.5.1.7 Relevant Literature on Bees

No relevant scientifically peer-reviewed open literature could be found on A14111B. Details of the literature search undertaken can be found in **M-CA Section 9**.

B.9.5.1.8 Residues in plant pollen and nectar

Report:	K-CP 10.3.1/01, North A, (2017). Chlorothalonil - Residue Study on Cucumber Pollen and Nectar in Northern and Southern France, Germany, Spain and Italy in 2015. Eurofins Agroscience Services LTD Report No. S15-03552. (Syngenta file No. A14111B/11654)
Previous evaluation	New study (submitted for the renewal)
RMS Comments	The study is reliable and can be used in the risk assessment.

Guidelines

EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees); EFSA Journal 2013; 11(7):3295.

Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document).

OECD Guidance Document on Crop Field Trials, Series on Pesticides No. 66 and Series on Testing and Assessment No. 164, ENV/JM/MONO(2011)50.

OECD Guidance Document on Overview of Residue Chemistry Studies (as revised 2009), Series on Testing and Assessment (No. 64) and Series on Pesticides (No. 32), ENV/JM/MONO(2009)31.

Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.

OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009.

OECD Series on Testing and Assessment No. 9 “Guidance document on the conduct of studies of occupational exposure to pesticides during agricultural application”, Paris 1997. OCDE/GD(97)148

European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000).

OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007).

The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO (2002) 9.

OECD Series on Principles of GLP and Compliance Monitoring No. 1 (as revised in 1997) "OECD Principles on Good Laboratory Practice", Paris 1998. ENV/MC/CHEM(98)17 and respective national regulations.

The national GLP requirements are based on the OECD Principles of Good Laboratory Practice, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and METI) on the basis of intergovernmental agreements.

GLP

Fully GLP compliant.

Executive Summary

Eight residue field trials on cucumber were conducted in Northern and Southern France, Germany, Spain and Italy during 2015.

Chlorothalonil and azoxystrobin were applied to the cucumber plants as A14111B, a suspension concentrate (SC) formulation containing 400 g/L of chlorothalonil and 80 g/L of azoxystrobin. Two applications, separated by a 6-8 day interval, one just before flowering (= application 1) and one during flowering (= application 2), were made at 1000 g chlorothalonil/ha and 200 g azoxystrobin/ha.

At trials S15-03552-01 and 02, untreated samples of pollen and nectar were collected 0 and 6 or 8days after application 2 (0 DAA2). Treated samples of pollen and nectar were collected 1 day after application 2 (1 DAA2), and at 6-10 DAA2.

At trials S15-03552-03 to 08, untreated and treated samples of pollen and nectar were collected 1 day after application 2 (1 DAA2) and at 7-9 DAA2.

The pollen and nectar samples were analysed for residues of chlorothalonil and its metabolite R182281. The analytical method GRM005.016A was validated for the determination of chlorothalonil and R182281 in pollen and nectar.

The ranges of residues of chlorothalonil and its metabolite R182281 in pollen and nectar from trials S15-03552-03 to S15-03552-08 are summarised in the table below.

Sampling Interval	Growth stage	Chlorothalonil	R182281
(days)	(BBCH)	Residues in the range	Residues in the range
		(mg/kg)	(mg/kg)

Treated Plot (P2): at a rate of 2 x 1000 g ai/ha			
Pollen			
1 DAA2	61 - 66	0.53 - 31	0.03 - 0.41
7-9 DAA2	61 - 79	0.03 - 2.5	<0.01 - 0.09
Nectar			
1 DAA2	61 - 66	<0.01 - 3.2	0.01 - 0.34
7-9 DAA2	61 - 79	<0.01 - 0.05	<0.01 - 0.04
Control plot (C1)			
No residues of chlorothalonil and R182281 at or above the limit of quantification (LOQ, 0.01 mg/kg) were found in the untreated samples, except for one pollen sample (S15-03552-04-009, 8 DALA), where a chlorothalonil residue of 0.04 mg/kg was detected. However, upon re-analysis, the residue was < 0.01 mg/kg.			

I. MATERIALS AND METHODS

A. MATERIALS

A1. Test Material

	A14111B
Description	Suspension concentrate formulation containing chlorothalonil and azoxystrobin
Batch number	GRA4K222B
Purity	407 g/L chlorothalonil; 82.4 g/L azoxystrobin
Stability of text compound	The test item is assumed to be stable for the period of use in the study, pending concurrent batch re-analysis

A2. Test Commodities

Crop:	Cucumber (Cucumis sativus)
Variety:	Tanja (S15-03552-01, S15-03552-02, S15-03552-04, S15-03552-06, S15-03552-07), Persica (S15-03552-03), Marketer (S15-03552-05, S15-03552-08),
Commodities:	Pollen and nectar

A3. Test Facilities

This study was performed at Eurofins Agroscience Services Ltd, Slade Lane, Wilson, Melbourne, Derbyshire DE73 8AG, United Kingdom; Eurofins Agroscience Services Chem GmbH, Grossmoorbogen 28, 21079 Hamburg; Eurofins Agroscience Services SAS, Z.I. des Sabotiers, F-49350 Gennes, France; Eurofins Agroscience Services SAS, 8 rue de la Collerette, F-45300 Rouvres St Jean, France; Eurofins Agroscience Services GmbH,Lempenseite 50/1, 69168 Wiesloch, Germany; Eurofins Agroscience Services GmbH, Lettenbödle 2, D-71706 Markgröningen, Germany; Eurofins Agroscience Services SRL, Zona Industriale, Strada XVIII 44, I-95121 Catania, Italy; Eurofins Agroscience Services SL,Serratella 18, E-46650 Canals, Valencia, Spain and Eurofins Agroscience Services SRL,Via Madonna delle Grazie 191°, I-04022 Fondi (Latina), Italy.

B. STUDY DESIGN

B1. Study Design

Eight residue field trials on cucumber were conducted in Northern and Southern France, Germany, Spain and Italy during 2015.

Chlorothalonil and azoxystrobin were applied to the cucumber plants as A14111B, a suspension concentrate (SC) formulation containing 400 g/L of chlorothalonil and 80 g/L of azoxystrobin. Two applications, separated by a 6-8 day interval, one just before flowering and one during flowering were made at 1000 g chlorothalonil/ha and 200 g azoxystrobin/ha.

At trials S15-03552-01 and 02, untreated samples of pollen and nectar were collected 0 (0 DAA2) and 6 or 8 days after application 2 (0 and 7±2 DAA2). Treated samples of pollen and nectar were collected 1 day after application 2 (1 DAA2), and at 7-10 DAA2. At trials S15-03552-01 to 08, untreated and treated samples of pollen and nectar were collected 1 day after application 2 (1 DAA2), and at 7-9 DAA2..

The samples were shipped frozen to the analytical laboratory for residue analysis.

B2. Analysis

The pollen and nectar samples were analysed for residues of chlorothalonil and its metabolite R182281. The analytical method GRM005.016A was validated for the determination of chlorothalonil and R182281 in pollen and nectar.

II. RESULTS AND DISCUSSION

Procedural recovery was 99% in pollen and 94% in nectar.

A summary of the measured residues from each trial is given in Tables 1.1 to 1.8

Table 1.1: Summary of chlorothalonil residues in pollen and nectar samples from trial S15-03552-01 conducted in France.

Sample No. S15-03552-01-	Number and Nominal Rate of Application (g ai/ha)*	Sampling Interval (days)	Crop	Residues (uncorrected)	
				Chlorothalonil	R182281
				(mg/kg)	(mg/kg)
Treated Plot					
005	2 x 1000	1 DAA2	Pollen	23	0.41
007			Nectar	0.20	0.08
013		9/10 DAA2	Pollen	0.35	0.04
013A			Pollen	0.83[1]	0.03
Mean:			Pollen	0.59	0.04
015			Nectar	0.01	<0.01
Control Plot					
001	Control	0 DAA2	Pollen	<0.01	<0.01
003			Nectar	<0.01	<0.01
009		8 DAA2	Pollen	<0.01	<0.01
011			Nectar	<0.01	<0.01

No correction of results for either control residues or recovery values has been performed.
*Application rate refers to chlorothalonil.
[1] Retain sample analysed to confirm initial result

Table 1.2: Summary of chlorothalonil residues in pollen and nectar samples from trial S15-03552-02 conducted in France.

Sample No. S15-03552-02-	Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (days)	Crop	Residues (uncorrected)	
				Chlorothalonil	R182281
				(mg/kg)	(mg/kg)
Treated Plot					
005	2 x 1000	1 DAA2	Pollen	2.2	0.08
006			Pollen	1.4[1]	0.03
Mean:			Pollen	1.8[2]	0.06
007			Nectar	<0.01	0.01
013		7 DAA2	Pollen	2.5	0.09
015			Nectar	<0.01	<0.01
Control Plot					
001	Control	0 DAA2	Pollen	<0.01	<0.01
002			Pollen	<0.01[3]	<0.01
003			Nectar	<0.01	<0.01
009		6 DAA2	Pollen	<0.01	<0.01
011			Nectar	<0.01	<0.01

No correction of results for either control residues or recovery values has been performed.
*Application rate refers to chlorothalonil.
[1] Retain sample analysed to confirm initial result
[2] Mean of two results for each analyte at the same sampling time point
[3] Used for fortification experiments

Table 1.3: Summary of chlorothalonil residues in pollen and nectar samples from trial S15-03552-03 conducted in Germany.

Sample No. S15-03552-03-	Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (days)	Crop	Residues (uncorrected)	
				Chlorothalonil	R182281
				(mg/kg)	(mg/kg)
Treated Plot					
005	2 x 1000	1 DAA2	Pollen	31	0.21
007			Nectar	0.06	0.02
013		9 DAA2	Pollen	not available	
015			Nectar	<0.01	<0.01
Control Plot					
001	Control	1 DAA2	Pollen	<0.01	<0.01
003			Nectar	<0.01	<0.01
009		9 DAA2	Pollen	not available	
011			Nectar	<0.01	<0.01

No correction of results for either control residues or recovery values has been performed.
*Application rate refers to chlorothalonil.

Table 1.4: Summary of chlorothalonil residues in pollen and nectar samples from trial S15-03552-04 conducted in Germany.

Sample No. S15-03552-04-	Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (days)	Crop	Residues (uncorrected)	
				Chlorothalonil	R182281
				(mg/kg)	(mg/kg)
Treated Plot					
005	2 x 1000	1 DAA2	Pollen	3.7	0.05
007			Nectar	2.2	0.21
013		8 DAA2	Pollen	0.25	0.01
015			Nectar	0.05	0.04
Control Plot					
001	Control	1 DAA2	Pollen	<0.01	<0.01
003			Nectar	<0.01	<0.01
009		8 DAA2	Pollen	<0.01[1]	<0.01
011			Nectar	<0.01	<0.01

No correction of results for either control residues or recovery values has been performed.
*Application rate refers to chlorothalonil.
[1] During initial analysis control sample S15-03552-04-009 showed a residue of 0.04 mg/kg. The same sample was re-analysed and showed a residue < 0.01 mg/kg. Multiple analysis of the retain sample S15-03552-04-010 yielded < 0.01 mg/kg (internal sample identification V16), < 0.01 mg/kg (internal sample identification V17). The initial value of 0.04 mg/kg was excluded since repeated analysis and duplicate analysis of the retain sample yielded residues < 0.01 mg/kg.

Table 1.5: Summary of chlorothalonil residues in pollen and nectar samples from trial S15-03552-05 conducted in Italy.

Sample No. S15-03552-05-	Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (days)	Crop	Residues (uncorrected)	
				Chlorothalonil	R182281
				(mg/kg)	(mg/kg)
Treated Plot					
005	2 x 1000	1 DAA2	Pollen	2.1	0.20
007			Nectar	0.26	0.20
013		7 DAA2	Pollen	0.12	<0.01
015			Nectar	0.02	<0.01
Control Plot					
001	Control	1 DAA2	Pollen	<0.01	<0.01
003			Nectar	<0.01	<0.01
009		7 DAA2	Pollen	<0.01	<0.01
011			Nectar	<0.01	<0.01

No correction of results for either control residues or recovery values has been performed.
*Application rate refers to chlorothalonil.

Table 1.6: Summary of chlorothalonil residues in pollen and nectar samples from trial S15-03552-06 conducted in Spain.

Sample No. S15-03552-06-	Number and Nominal Rate of Application	Sampling Interval (days)	Crop	Residues (uncorrected)	
				Chlorothalonil	R182281

	(g ai/ha)			(mg/kg)	(mg/kg)
Treated Plot					
005	2 x 1000	1 DAA2	Pollen	0.53	0.04
007			Nectar	0.08	0.02
013		8 DAA2	Pollen	0.03	<0.01
015			Nectar	<0.01	<0.01
Control Plot					
001	Control	1 DAA2	Pollen	<0.01	<0.01
003			Nectar	<0.01	<0.01
009		8 DAA2	Pollen	<0.01	<0.01
011			Nectar	<0.01	<0.01

No correction of results for either control residues or recovery values has been performed.
*Application rate refers to chlorothalonil.

Table 1.7: Summary of chlorothalonil residues in pollen and nectar samples from trial S15-03552-07 conducted in Spain.

Sample No. S15-03552-07-	Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (days)	Crop	Residues (uncorrected)	
				Chlorothalonil	R182281
				(mg/kg)	(mg/kg)
Treated Plot					
005	2 x 1000	1 DAA2	Pollen	5.1	0.07
007			Nectar	0.57	0.06
013		8 DAA2	Pollen	0.30	0.01
015			Nectar	0.02	<0.01
Control Plot					
001	Control	1 DAA2	Pollen	<0.01	<0.01
003			Nectar	<0.01	<0.01
009		8 DAA2	Pollen	<0.01	<0.01
011			Nectar	<0.01	<0.01

No correction of results for either control residues or recovery values has been performed.
*Application rate refers to chlorothalonil.

Table 1.8: Summary of chlorothalonil residues in pollen and nectar samples from trial S15-03552-08 conducted in Spain.

Sample No. S15-03552-08-	Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (days)	Crop	Residues (uncorrected)	
				Chlorothalonil	R182281
				(mg/kg)	(mg/kg)
Treated Plot					
005	2 x 1000	1 DAA2	Pollen	7.0	0.17
007			Nectar	3.2	0.34
013		7 DAA2	Pollen	0.31	0.04
015			Nectar	0.02	0.01
Control Plot					

001	Control	1 DAA2	Pollen	<0.01	<0.01
003			Nectar	<0.01	<0.01
009		7 DAA2	Pollen	<0.01	<0.01
011			Nectar	<0.01	<0.01

No correction of results for either control residues or recovery values has been performed.
*Application rate refers to chlorothalonil.

III. CONCLUSION

This section discusses uncorrected results.

Analysis of field samples

Eight residue field trials on cucumber were conducted in Northern and Southern France, Germany, Spain and Italy during 2015.

Chlorothalonil and azoxystrobin were applied to the cucumber plants just before flowering and during flowering as A14111B, a suspension concentrate (SC) formulation containing 400 g/L of chlorothalonil and 80 g/L of azoxystrobin. Two applications, separated by a 6-8 day interval, were made at 1000 g chlorothalonil/ha and 200 g axoxystrobin/ha.

At trials S15-03552-01 and 02, untreated samples of pollen and nectar were collected 0 and 6 or 8 days after application 2 (0, 7±2 DAA2). Treated samples of pollen and nectar were collected 1 day after application 2 (1 DAA2), and at 7-10 DAA2.
At trials S15-03552-03 to 08, untreated and treated samples of pollen and nectar were collected 1 day after application 2 (1 DAA2), and at 7-9 DAA2

The pollen and nectar samples were analysed for residues of chlorothalonil and its metabolite R182281.
The analytical method GRM005.016A was validated for the determination of chlorothalonil and R182281 in pollen and nectar.

The study design as detailed above was successfully carried out leading to the following conclusions. Residues of chlorothalonil and its metabolite R182281 are summarized in the table below.

Sampling Interval (days)	Growth stage (BBCH)	Chlorothalonil Residues in the range (mg/kg)	R182281 Residues in the range (mg/kg)
Treated Plot (P2): at a rate of 2 x 1000 g ai/ha			
Pollen			
1 DAA2	61 - 66	0.53 - 31	0.03 - 0.41
7-9 DAA2	61 - 79	0.03 - 2.5	<0.01 - 0.09
Nectar			
1 DAA2	61 - 66	<0.01 - 3.2	0.01 - 0.34
7-9 DAA2	61 - 79	<0.01 - 0.05	<0.01 - 0.04
Control plot (C1)			
No residues of chlorothalonil and R182281 at or above the limit of quantification (LOQ, 0.01 mg/kg) were found in the untreated samples except for one pollen sample (S15-03552-04-009, 8 DALA), where a chlorothalonil residue of 0.04 mg/kg was detected. However, upon re-analysis, the residue was < 0.01 mg/kg.			

Study Comments: KCP 10.3.1/01	No comments
Agreed Endpoint(s): KCP 10.3.1/01	

Sampling Interval (days)	Growth stage (BBCH)	Chlorothalonil Residues in the range (mg/kg)	R182281 Residues in the range (mg/kg)
Treated Plot (P2): at a rate of 2 x 1000 g ai/ha			
Pollen			
1 DAA2	61 - 66	0.53 - 31	0.03 - 0.41
7-9 DAA2	61 - 79	0.03 - 2.5	<0.01 - 0.09
Nectar			
1 DAA2	61 - 66	<0.01 - 3.2	0.01 - 0.34
7-9 DAA2	61 - 79	<0.01 - 0.05	<0.01 - 0.04
Control plot (C1)			
No residues of chlorothalonil and R182281 at or above the limit of quantification (LOQ, 0.01 mg/kg) were found in the untreated samples except for one pollen sample (S15-03552-04-009, 8 DALA), where a chlorothalonil residue of 0.04 mg/kg was detected. However, upon re-analysis, the residue was < 0.01 mg/kg.			

North, A.	2017	Chlorothalonil - Residue Study on Cucumber Pollen and Nectar in Northern and Southern France, Germany, Spain and Italy in 2015.	Eurofins Agroscience Services LTD Report No. S15-03552. (Syngenta file No. A14111B/11654)

Reliability	
General information	
Is a guideline method or modified guideline used?*	y
Is the test performed under GLP conditions?*	y

If applicable, are validity criteria fulfilled (e.g. control survival, growth, etc.)?	Not relevant for residue study
Are appropriate controls performed (e.g. solvent control, negative and/or positive control)?	y
* these criteria are of minor importance for study reliability, but may support study evaluation	
Test compound	
Is the test substance clearly identified with name or CAS-number? Are test results reported for the appropriate compound?	y
Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	y
If a formulation is used or if impurities are present: do other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	nr
Test organism	
Are the organisms well described (e.g. scientific name, weight, length, growth, age/life stage, strain/clone, gender if appropriate)?	y
Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?	Y n
Exposure conditions	
Is the experimental system appropriate for the test substance, taking into account its physicochemical characteristics?	y
Is the experimental system appropriate for the test organism? Have conditions been stable during the test?	y
If appropriate, were exposure concentrations below the limit of water solubility (taking the use of a solvent into account)? If a solvent is used, is the solvent within the appropriate range and is a solvent control included?	Not reported
Is a correct spacing between exposure concentrations applied?	nr
Is the exposure duration defined?	y
If necessary, are chemical analyses adequate to verify concentrations of the test substance over the duration of the study?	Not relevant
Where applicable, is the biomass loading of the organisms in the test system within the appropriate range?	y
Statistical Design and Biological Response	
Is a sufficient number of replicates used? Is a sufficient number of organisms per replicate used for all controls and test concentrations?	nr

Are appropriate statistical methods used?	-
Is a concentration-response curve observed? Is the response statistically significant?	nrnr
Are sufficient data available to check the calculation of endpoints and (if applicable) validity criteria (e.g., control data, concentration-response curves)?	y
Relevance of the study for Environmental Risk Assessment, appropriateness of study endpoints	
Exposure Relevance	
Is the substance tested representative and relevant for the substance being assessed?	y
Is the tested exposure scenario relevant for the substance?	y
Is the tested exposure scenario relevant for the species?	y
Biological relevance	
Is the species tested relevant for the compartment under evaluation?	Not applicable
Are the organisms tested relevant for the tested compound?	Not applicable
Are the reported endpoints appropriate for the regulatory purpose?	Not applicable
Are the reported endpoints appropriate for the investigated effects or the mode of action of the test substance?	Not applicable
Is the effect relevant on a population level?	Not applicable
Is the magnitude of effect statistically significant and biologically relevant for the regulatory purpose (e.g. EC10, EC50)?	Not applicable
Are appropriate life-stages studied?	Not applicable
Are the experimental conditions relevant for the tested species?	Not applicable
Is the exposure duration relevant and appropriate for the studied endpoints and species?	Not applicable
If recovery is studied, is this relevant for the framework for which the study is evaluated?	Not applicable
Concluding weight of evidence/proposed action	To be used for risk assessment
Type of information (Fully acceptable, supporting information, not applicable)	Fully acceptable
Consideration/concluding score	R1, C1

B.9.5.2 Effects on non-target arthropods other than bees

Report:	IIIA, 10.5/01 (numbering in addendum 09 of the DAR (2001)). Vinall, S., 2000. Chlorothalonil 720 g/l SC (YF10938): A laboratory test to determine effects on the predatory mite, *Typhlodromus pyri* (phytosiidae). Generated by: Agrochemical Evaluation Unit. Submitted by: Zeneca Company file: 41290 date: 27 January 2000
Previous evaluation	In addendum 09 of the DAR (2001) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Test type	Conditions	Dosage kg a.i./ha	Criterion	Effect	Unit	Classification	Ri
Bravo 720 g/l SC	Typhlodromus pyri	Contact	Laboratory	7.7 6 1.5	Reproduction	94 94 78	%	Harmfull	1

Description

Protonymph mites (20 per replica, three replicas per treatment) were placed onto petri dishes treated with three rates of chlorothalonil 720 g/l SC (7.7, 6, and 1.5 kg a.i./ha). Mites were fed with pollen and their survival assessed over a period of 7 days. Survivors were then transferred to fresh petri dishes that were treated at the same time as the first set. At least one male per five females were transferred. Mites were fed pollen every 1-3 days. Units were kept under controlled conditions 20-24 ºC, 42-75% humidity with a 16h. photoperiod. Their fecundicity was assessed over a further 7 days. Dimethoate was used as toxic reference substance.

Results

A control corrected mortality of 5% was measured at the highest test concentration. Using Tukey's test the mean number of eggs produced per female was found te be different from the control for the 6 and 7.7 kg a.i./ha but not for the 1.5 kg a.i./ha. Recalculation with other test like Dunnett's and Williams shows a significant difference for the 1.5 kg a.i./ha dosage as well.

Remarks

The result in the heading table is used for risk evaluation.

Report:	IIIA, 10.5/02 (numbering in addendum 09 of the DAR (2001)). Sankanu, A., 2000. A Tier I laboratory study study to evaluatethe effects of Chlorothalonil (YF10938) on the green lacewing, *Chrysoperla carnea*. Generated by: Ecotox limited. Submitted by: Zeneca Company file: 41292 date: 14 March 2000
Previous evaluation	In addendum 09 of the DAR (2001) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Test type	Conditions	Dosage kg a.i./ha	Criterion	Value	Unit	Classification	Ri
Bravo 720 g/l SC	Chrysoperla carnea	Contact	Laboratory	0.31 6 7.67	Combined effect	11 16 2.7	%	Harmless	1

Description

Lacewing larvae (8 per replica, 5 replicas per treatment) were placed onto glass plates treated with three rates chlorothalonil 720 g/l SC (310, 6000 and 7670 g a.i./ha); purity 52.7% w/w. *Ephistia* spp.eggs were added to each test unit as food. Units were kept under controlled conditions at 22 - 24ºC, 54-66% humidity with a 16h. photoperiod. Dimethoate was used as toxic reference substance. Mortality was assessed 1, 3, 5, 7, 9, 11 and 13 days after treatment. Once surviving larvae had pupated they were transferred to untreated culturing chambers, one chamber for each treatment replicate. Fecundicity assessment started 8 days after the first eggs were observed in the control. All old egg were removed and the number of males and females recorded. Egg production was assessed twice over a one week period and each assessment covered a 24h period. The number of eggs produced was recorded along with the number of males and females to determine the number of eggs per female per 24h period. A number of eggs was further assessed for hatching rate. The number of hatched larvae was recorded daily.

Results

A corrected mortality of 8% was measured at 0.3 and 6 kg/ha dose and 5% at the 7.7 kg/ha dose. Using Dunnet's test the mean number of eggs produced per female did not differ significantly from the control for the tested concentrations. The mean number of eggs produced was 2.7% less for 0.3 kg/ha; 5.4% less for 6 kg/ha and 2% more for 7.7 kg/ha dose. The mean number of hatched eggs per female did not significantly differ from the control, it was 2.9% lower for 0.3 kg/ha; 10.3% lower for 6 kg/ha and 4.9% lower for 7.7 kg/ha dose.

Remarks

The result in the heading table is used for risk evaluation.

Report:	IIIA, 10.5/03 (numbering in addendum 09 of the DAR (2001)). Baxter, I., 2000. Chlorothalonil 720 g/l SC (YF10938): A laboratory study to determine the effects on parasitoid, *Aphidius rhopalosiphi.* Generated by: Agrochemical Evaluation Unit. Submitted by: Zeneca Company file: 41291 date: 31 March 2000
Previous evaluation	In addendum 09 of the DAR (2001) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Test type	Conditions	Dosage kg a.i./ha	Criterion	Value [%]	classification	Ri
Bravo 720 g/l SC	Aphidius rhopalosiphi	Contact	Laboratory	0.044	Total effect	5	Harmless	1
				0.173	mortality	55	Moderately harmful	
				1.1	mortality	41	Moderately harmful	
				4.3	mortality	62	Moderately harmful	
				7.7	mortality	55	Moderately harmful	

Description

Test methods were based on the guideline of Mead-Briggs. Glass plates were treated with five rates chlorothalonil 720 g/l SC (44, 173, 1100, 4333 and 7700 g a.i./ha); content 52.7% w/w. Ten adult wasps (including a minimum of five females) were placed in each replica arena (3 replicas per treatment). Pieces of cotton wool soaked with a honey water solution were administered as food source. Units were kept under controlled conditions at 19- 22ºC, 67-74% humidity and with a 16h. photoperiod. Dimethoate was used as toxic reference substance.

The conditon of the wasps was assessed at approx. 24 and 48 hours after treatment. After 48 hours fecundicity assessment has been carried out for the control and the concentration with a mortality of no more than 10%. 15 Female wasps were transferred to pots of barley seedlings infested with host aphids. After 24h. the adult wasps were removed and the infested plants were kept under similar conditions for 11 days before the number of aphid mummies was recorded.

Results

Corrected mortalities for treatment concentrations >44g a.i./ha are reported in the header. At 44 g a.i./ha a corrected mortality of 10% was calculated. There was an increase of 5% in the number of aphid mummies measured in this treatment compared to the control. The calculated overall effect on beneficial capacity was 5%.

Remarks

The effect based on mortality is between 41 and 62% for the doses > 44 g a.i./ha, there is no effect on beneficial capacity at the lowest dose of 0.044 kg a.i./ha. The result in the heading table is used for risk evaluation.

Report:	IIIA, 10.5/04 (numbering in addendum 09 of the DAR (2001)). Baxter, I., 2000. Chlorothalonil 720 g/l SC (YF10938): A laboratory test to determine effects on the ground beetle, *Poecilus cupreus.* Generated by: Agrochemical Evaluation Unit. Submitted by: Zeneca Company file: 41289 date: 31 March 2000
Previous evaluation	In addendum 09 of the DAR (2001) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Test type	Conditions	Dosage kg a.i./ha	Criterion	Value	Unit	Classification	Ri
Bravo 720 g/l SC	Poecilus cupreus	Contact	Laboratory	10.5	Effect	0	%	Harmless	1

Description

Test methods were based on the guideline of Heimbach (1991). Adult beetles were treated exposed on the surface of boxes with damp sand.The treatment rate chlorothalonil 720 g/l SC (a.i. content 52.7% w/w) was 10.5 kg a.i./ha; Five replicate boxes for each treatment (treatment, control and toxic reference), three male and three female beetles per box. Assessment on condition and feeding activity. One fly pupae per live beetle were administered on five occassions. The boxes were kept in a controlled environment room at 19- 22ºC, 34-74% humidity and with a 16h. photoperiod. Dimethoate was used as toxic reference substance.

Results

None of the beetles died in the control nor in the chlorothalonil treatment. 100% mortality in the toxic reference within 4 days. The feeding activity in the chlorothalonil treatment was not significantly different from the control. Control beetles consumed 1.03 pupae each compare to 0.85 in the lower rate test substance and 1.28 in the higher rate.

Remarks

There is no effect on survival or feeding activity of the ground beetle. According to the EPPO/IOBC classification for a worst-case laboratory test chlorothalonil is harmless to ground beetle at a dose concentration of 10.5 kg a.i./ha. The results are used for risk evaluation.

Report:	IIIA, 10.5/05 (numbering in addendum 14 of the DAR (2004)). Baxter, I., 2000. Chorothalonil: a Tier II extended laboratory study to evaluate the effects of a 720 g/L SC formulation on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) Report No. ZEN-00-14/C

Previous evaluation	In addendum 14 of the DAR (2004) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Method	Dose [kg as/ha]	Exposure duration [h]	Parameter	Value [%]	After …
720 g/L SC formulation (BRAVO 720)	*Aphidius rhopalosiphi*	Residues on barley seedlings	4.33; 7.70; 18.75	48	Mortality Fecundity	20; 20; 44 no significant effects at dosages up to 7.70 kg as/ha (at 18.75 kg as/ha the effects of fecundity are not assessed)	48 h 12 d

Description

The effects of Chlorothalonil 720 g/L SC (BRAVO 720) on survival of the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) after 48 hours of exposure were determined in an extended laboratory test under GLP. In addition, a record was made of any significant sub-lethal treatment effects of the fecundity of wasps at the highest two treatment rate at which no apparent acute harmful effects were seen.The test design was in line with current European testing guidelines (Barrett et al., 1994).

Following an initial range-finding test, chlorothalonil 720 g/L SC was evaluated in a definitive test at three separate treatment rates, nimnally equivalent to 18.75, 7.70 and 4.33 kg a.s./ha (26.04, 10.70 and 5.89 L product/ha). The effects of these treatments were compared to a toxic reference of BASF Dimethoate 40 EC, applied at a rate of 8.5 mL product/ha (3.4 g a.s./ha), and a control of deionised water. All treatments were applied to pots of seedling barley at a rate equivalent to 200 L spray solution/ha. Once they had dried, the pots of plants were enclosed within cylindrical ventilated collars. For the definitive test, five female wasps were confined over each pot, with five replicates (25 wasps) prepared for each treatment. The behaviour of the wasps was assessed during the initial 2,5 h to determine whether there was any apparent repellence from the treated plants. Was survival was assessed over a period of 48 h. To determine any sub-lethal treatment effects on the fecundity of the surviving insects, female wasps (n = 15 per treatment) were individually confined over pots of untreated, aphid-infested barley plants for 24 h and then removed. The number of parasitised aphid mummies that developed on each pot of plants was recorded 12 days later.

Results

The percentage mortality for the treatment rates of 4.33, 7.70 and 18.75 kg a.s./ha was respectively 20, 20 and 44%. All of the wasps in the toxic reference treatment were dead at 24 h.The treatment rates of 4.33 and 7.70 kg a.s./ha had no significant effects on fecundity; the mean number of aphids parasitised per female over 24 h was 15.1, 16.3 and 15.6 for respectively the control and the treatment rates of 4.33 and 7.70 kg a.s./ha. For the highest treatment rate (18.75 kg a.s./ha) the effects on fecundity were not assessed.

There was a weak repellence effect at all treatment rates of chlorothalonil 720 g/L SC.

Remarks by RMS

The results are used for the risk assessment.

Report:	IIIA, 10.5/06 (numbering in addendum 14 of the DAR (2004)). Vinall, S., 2000. Chorothalonil: a Tier II extended laboratory study to evaluate the effects of a 720 g/L SC formulation on the predatory mite, *Typhlodromus pyri* (Acarina, Phytoseiidae) Report No. ZEN-00-15/C
Previous evaluation	In addendum 14 of the DAR (2004) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	**Species**	**Method**	**Dose** [kg as/ha]	**Exposure duration** [d]	**Parameter**	**Value** [%]	**After …** [d]
720 g/L SC formulation (BRAVO 720)	*Typhlodromus pyri*	Residues on sprayed leaf discs	1.50; 1.88; 5.63; 12.00; 18.75	7	Mortality	0; 0; 9; 17 ; 13	7

Description

The effect of Chlorothalonil 720 g/L SC (BRAVO 720) on survival and fecundity of the predatory mite *Typhlodromus pyri* Scheuten after 7+7 days of exposure was determined in an extended laboratory test under GLP. In addition, a record was made of any significant sublethal treatment effects on the subsequent fecundity of the mites.The test design was in line with current European testing guidelines (Barrett et al., 1994).

Following a range-finding test, chlorothalonil 720 g/L SC was evaluated in a definitive test at five treatment rates, nominally equivalent to 18.75, 12.00, 5.625, 1.875 and 1.50 kg a.s./ha. The effects of these treatment were compared to a toxic reference of BASF Dimethoate 40 EC, applied at a rate of 212.5 mL product/ha (85 g a.s./ha), and a control fo deionised water.

For the definitive test, treatments were applied to excised leaf discs (n=3 per treatment) taken from dwarf French bean plants. The treated leaf discs were then laid onto water-saturated cotton wool and arenas created on their surface using a ring of non-drying sticky gel. Protonymphal mites (20 per replicate) were then placed within the arenas and were provided daily with untreated bean pollen for food. Their survival was assessed over a 7-day period. At 7 days the adult mites from the control and from all of the treatment rates of the test item were transferred to untreated glass plates. The individual replicates were maintained for each treatment and the sex of the mites was noted. A barrier of non-drying sticky gel was again used to confine the mites and they were fed daily with untreated bean pollen. The egg production in each arena was assessed between 7 and 14 days after treatment (DAT) so that the mean number of eggs produced per female could be calculated for each treatment.

Results

The corrected percentage mortality for the treatment rates of 1.50, 1.88, 5.63, 12.00 and 18.75 kg a.s./ha was respectively 0, 0, 9, 17 and 13%. All of the wasps in the toxic reference treatment were dead at 7 days.

All of the treatment rates reduced the rate of juvenile mite development, such that the onset of egg production was delayed. The mean number of eggs per female (7 – 14 DAT) were 8.6, 5.0, 4.6, 3.8, 4.4 and 3.1 for respectively the control and the treatment rates of 1.50, 1.88, 5.63, 12.00 and 18.75 kg a.s./ha. The NOER (No Observed Effect Rate) was therefore determined to be < 1.50 kg a.s./ha.

Remarks by RMS

The results are used for the risk assessment.

Report:	IIIA, 10.5/07 (numbering in addendum 14 of the DAR (2004)). Wainwright, S., 2003. Chlorothalonil 75 WG. An extended laboratory test to evaluate the effects of pesticides on adults of the cereal aphid parasitoid *Aphidius rhopalosiphi* Report No. VCM 107/023414.
Previous evaluation	In addendum 14 of the DAR (2004) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	**Species**	**Method**	**Dose**	**Exposure duration**	**Parameter**	**Value**	**After …**
			[kg as/ha]	**[h]**		**[%]**	
Chlorothalonil 75 WG	*Aphidius rhopalosiphi*	Residues on barley seedlings	0.47; 1.5; 5.4	48	Mortality Fecundity Overall effect	16.7; 43.3; 63.3 R* = 40; 50; 64 E = 50; 72; 87	48 h 11 d

* R: Reduction (%) in reproduction

Description

The effects of Chlorothalonil 75 WG (batch no. B111, a 750 g/L formulation of chlorothalonil) on survival and fecundity of the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) after 48 hours of exposure were determined in an extended laboratory test under GLP based upon the method outlined by Mead-Briggs *et al*. (2000), in the IOBC, BART and EPPO Joint Initiative and in accordance with ESCORT 2 (Candolfi *et al*., 2001).

Adult wasps (< 48 hours old mated females) were exposed to fresh, 1 h dried residues of the test substance sprayed on barley seedlings (10 per pot) at three dose levels: 0.47, 1.5 and 5.4 kg as/ha with an actual spray volume of 400 L/ha water. A water control (400 L/ha) and a toxic standard (Dimethoate 400 g/L EC at 10 g as/ha in 400 L /ha water) were included in the test. A calibrated Potter laboratory precision spray tower was used. Thirty female wasps were used per treatment, with 6 replicates of five wasps. Exposure chambers (plant pots, 10 cm diameter) were covered by acrylic cylinders with a fine mesh covering and a hole in the side for the introduction of the wasps. Feed: 10% (w/v) fructose solution sprayed on the plants (and dried) before application of test treatment and 1:3

honey: water solution on cotton wool at the end of the position assessments. Environmental conditions: 19-25 °C, 59-94% RH and daily 16 hours light (exposure phase: 1132 lux, fecundity phase: 3550-5800 lux). Wasps were observed every half-hour from ½ h to 2½ h after treatment for abnormal behaviour (position assessment) and after 2 h, 24 h and 48h for condition (alive, affected, moribund or dead). Fecundity assessments were made for 15 surviving females per treatment, except the 5.4 kg as/ha treated group (only 8 survivors) and the toxic reference (no survivors). Wasps were individually enclosed for 24 h in chambers with 3 seedlings infested with 70 *Rhopalosiphium padi* nymphs. After 11 days numbers of parasitized aphids per chamber were assessed.

Statistics used: F1 test for monotonicity (Healey, 1999), Williams (1971, 1972) for position assessments and parasitisation rate, Student's *t* test for the control groups.

Results

No abnormal behaviour occurred at any test substance concentration compared to the control, except for the toxic reference (wasps moved from the plants to the sand and became affected).

Mean cumulative mortality (including moribund wasps) after 48 hours was 16.7%, 43.3% and 63.3% at 0.47, 1.5 and 5.4 kg as/ha, respectively. No mortality was found in the blank control. Mortality in the positive control was 100% within 24 hours.

The mean numbers of parasitized aphids per female at 0, 0.47, 1.5 and 5.4 kg as/ha were 33.33, 20.0, 16.73 and 12.13, respectively (percentages of parasitized aphids: 47.6%, 28.6%, 23.9% and 17.3%, respectively). Reductions in the treated groups were significant ($P < 0.05$ at 0.47kg as/ha and $P < 0.01$ at the higher rates) as compared to the untreated control.

The reduction of reproduction found in the treatment groups at 0.47, 1.5 and 5.4 kg as/ha compared to the control was 40%, 50% and 64%, respectively.

Remarks by RMS

Overall effects were calculated by RMS using the formula of Overmeer and Van Zon. The E values are 50%, 72% and 85% for the 0.47, 1.5 and 5.4 kg as/ha rate, respectively.

The results on mortality (16.7%, 43.3% and 63.3%), reduction of reproduction (40%, 50% and 64%) and the E values (50%, 72% and 85%) for the 0.47, 1.5 and 5.4 kg as/ha rate, respectively are used for risk assessment.

Report:	IIIA, 10.5/08 (numbering in addendum 14 of the DAR (2004)). Wainwright, S., 2003. Chlorothalonil 500 SC. An extended laboratory test to evaluate the effects of pesticides on the predacious mite *Typhlodromus pyri*. Report No. VCM 108/023416
Previous evaluation	In addendum 14 of the DAR (2004) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Method	Dose	Exposure duration	Parameter	Value	After …

			[kg as/ha]	[d]		[%]	[d]
Chlorothalonil 500 SC	*Typhlodromus pyri*	Residues on sprayed leaf discs	0.047; 1.5; 5.4	7	Mortality Fecundity	0; 12.25; 9.18 R*=20, 70; 75	7 14

* R: Reduction (%) in reproduction

Description

The effect of Chlorothalonil 500 SC (batch no. B110, a 500 g/L SC formulation of chlorothalonil) on survival and fecundity of the predatory mite *Typhlodromus pyri* Scheuten after 7+7 days of exposure was determined in an extended laboratory test under GLP based on methods of Louis and Ufer (1995) and in accordance with methods of Blumel *et al*.(2000) in: Candolfi *et al.* (2000), IOBC, BART and EPPO Joint Initiative. Rates used were calculated in accordance with Candolfi *et al*. (ESCORT 2, 2001). Protonymphs (~ 24 hours old) were exposed to fresh (about 1 hour after application), dried residues of the test substance sprayed on 4 cm diameter leaf discs from French bean plants at three dose levels: 0.047, 1.5 and 5.4 kg as/ha with an actual spray volume of 400 L/ha water. A water control (400 L/ha) and a toxic standard (Dimethoate 400 g/L EC at 20 g as/ha in 400 L /ha water) were included in the test. A calibrated Potter laboratory precision spray tower was used. Twenty mites were used per replicate, with 5 replicates per treatment. Feed (fresh dwarf bean pollen) was provided *ad libitum*. Environmental conditions: 24-26 °C, 66-87% RH and daily 16 hours light (1344-1796 lux). Observations on behaviour and mortality were performed on days 3 and 7. On day 7 the number of male and female mites was counted. On days 9, 11 and 14 the numbers of surviving adults and the total number of eggs laid and hatched juveniles were determined.

Statistics used: calculations of corrected mortality (including escaped mites) according to Schneider-Orelli. F1 test for monotonicity (Healey, 1999), Williams (1971, 1972) for position assessments and parasitisation rate, Student's *t* test for the control groups.

Results

On day 7 control mortality was 2%; corrected mortality in the positive control was 96.9%. Mean corrected mortality in the treated groups after 7 days was 0%, 12.25% and 9.18% at 0.047, 1.5 and 5.4 kg as/ha, respectively. Fecundity was significantly reduced on days 9, 11 and 14 in the 1.5 and 5.4 kg as/ha treated groups. The mean cumulative number of eggs per female was 8.57 in the water control and 6.89, 2.54 and 2.14 in the 0.047, 1.5 and 5.4 kg as/ha treated groups. Cumulative percentage reduction in reproduction compared to untreated control was 20%, 70% and 75% at 0.047, 1.5 and 5.4 kg as/ha, respectively. The 9-, 11- and 14-day EC_{50} (95% CI) values based on fecundity were calculated to be 0.094 (95% CI: 0.027-0.324), 0.276 (0.053-1.439) and 1.555 (0.497-4.870), respectively.

Remarks by RMS

The EC_{50} is not used for risk assessment because only 3 rates are available.

Overall effects were calculated by RMS using the formula of Overmeer and Van Zon. The E values are 20%, 74% and 77% for the 0.047, 1.5 and 5.4 kg as/ha rate, respectively.

The results on mortality (0%, 12.25% and 9.18%), reduction of reproduction (20%, 70% and 75%) and the E values (20%, 74% and 77%) for the 0.047, 1.5 and 5.4 kg as/ha rate, respectively are used for risk assessment.

Report	K-CP 10.3.2.1/01 Fussell S. (2004) Azoxystrobin and chlorothalonil: A rate-response laboratory test to evaluate effects of an 80 + 400 g/L SC formulation (A14111B) on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae).. Report Number SYN-03-34, Mambo-Tox Ltd, Southampton, UK. (Syngenta File No. ICI5504/2214)
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. The LR_{50} is > 625 mL A14111B/ha (corresponding to > 262 g chlorothalonil/ha). The ER_{50} is > 2500 mL A14111B/ha (corresponding to > 1048 g chlorothalonil /ha).

Guidelines

Mead-Briggs *et al*. (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae).

GLP: Yes.

Executive Summary

Azoxystrobin/chlorothalonil SC (80/400) is a suspension concentrate (SC) formulation (hereafter referred to as A14111B) nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil. The aim of this study was to determine, under worst-case laboratory test conditions, the effects of A14111B on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae).

Following an initial range-finding test, A14111B was evaluated in a definitive test at five application rates, equivalent to 5000, 2500, 1250, 625 and 312.5 mL A14111B/ha. Also included in the definitive test were a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 0.20 mL product/ha (0.08 g a.i./ha). Treatments were applied to glass plates that were then used to form the floor and ceiling of shallow arenas. Ten adult wasps (including a minimum of five females) were placed in each replicate arena (n = 4 per treatment rate). Assessments of treatment effects were made over 48 h.

The mortality in the control treatment at 48 h was 10%. This compared with mortalities of 55%, 48%, 63%, 25% and 28% in the 5000, 2500, 1250, 625 and 312.5 mL product/ha treatment rates of A14111B, respectively, and 60% in the toxic reference treatment. Corrected mortalities in the respective test item treatments were 50%, 42%, 58%, 17% and 19%.

An assessment of the subsequent reproductive capacity of individually-confined females was performed at the three highest rates that had resulted in a < 50% corrected mortality (i.e. 312.5, 625 and 2500 mL/ha).

In the reproduction assessments, the mean number of mummies produced per surviving female was 82.7, compared with 68.5, 67.0 and 88.0 mummies per surviving female in the 2500, 625 and 312.5 mL product/ha treatment rates, respectively. The mean numbers of mummies per female was not significantly affected in any of the treatment rates tested (ANOVA, P > 0.05).
In conclusion, no clear rate-response relationship was observed in respect of mortality, but treatment rates of 1250, 2500 and 5000 mL A14111B/ha did have statistically significant effects on wasp survival. The reproductive performance of surviving wasps was not significantly affected at any of the treatment rates evaluated (i.e. 2500, 625 and 312.5 mL A14111B/ha).

Materials

Test Material:	Azoxystrobin/chlorothalonil SC (80/400) (formulation code A14111B)
Description:	Opaque cream-coloured suspension concentrate, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil
Lot/Batch #:	J7518/024
Purity:	80 g/L azoxystrobin (corresponding to 6.6%) and 419 g/L (corresponding to 34.6%) chlorothalonil
Stability of test compound:	Assumed stable pending re-analysis in September 2005
Vehicle and control:	Deionised water
Toxic reference:	Perfekthion (400 g dimethoate/L) in deionised water (0.2 mL product/ha)
Spray volume rate:	200 L spray solution/ha
Application method:	Potter Laboratory Spray Tower, calibrated for each treatment preparation.
Test organisms	
Species:	*Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae)
Source:	Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*).
Food:	1:3 v/v solution of honey in water
Test substrate:	Glass plates
Environmental test conditions	
Temperature:	Mortality assessment phase: 19 to 22°C Fecundity assessment phase: 20 to 21°C
Humidity:	Mortality assessment phase: 65 to 87% relative humidity
Photoperiod:	Mortality assessment phase: 16 h photoperiod (1200-1500 lux) Fecundity assessment phase: 16 h photoperiod (4900-5200 lux)

Study Design and Methods

Experimental dates: 6th January to 10th February 2004.
Treatments were applied to glass plates that were then used to form the floor and ceiling of shallow arenas. Ten adult wasps (including a minimum of five females, < 48 hours old) were placed in each replicate arena (n = 4 per treatment rate). Assessments of treatment effects were made over 48 h. To assess any sub-lethal effects, reproduction assessments were then carried out for the control and from the three highest treatment rates of the test item that had resulted in < 50% mortality. Up to fifteen female wasps were confined individually for 24 h over untreated barley plants previously infested with cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). The wasps were then removed and the plants left for a further 10 days before the number of 'mummies' (parasitised aphids containing wasp pupae) that had developed was recorded.

Results and Discussion

The results of the mortality assessments are summarised in Table 10.3.2.1-1. At 48 h, mortality in the 5000, 2500 and 1250 mL/ha treatment rates differed significantly from the control (Fisher's Exact Test, P < 0.001). No clear rate-response relationship was observed in relation to mortality.

Table 10.3.2.1-1: Effects of fresh dry residues of A14111B on mortality of *Aphidius rhopalosiphi*, when exposed under laboratory test conditions

Treatment	Rate (mL/ha)	% mortality at 48 h [a]	Corrected % mortality
Control		10	-
A14111B	5000	55*	50
	2500	48*	42
	1250	63*	58
	625	25	17
	312.5	28	19
Perfekthion	0.2	60*	56

[a] The percentage mortality in each treatment was compared to that in the control using Fisher's Exact Test. Treatment means marked with asterisks differed significantly from the control (* P < 0.001).

The results of the reproduction assessments are summarised in Table 10.3.2.1-2. The performance of the surviving wasps was not significantly affected at any of the treatment rates evaluated (i.e. 2500, 625 and 312.5 mL A14111B/ha).

Table 10.3.2.1-2: Effects of fresh dry residues of A14111B on the reproductive capacity of *Aphidius rhopalosiphi*, when exposed under laboratory test conditions

Treatment	Rate (mL/ha)	Mean number mummies per surviving female [a]	Standard deviation	% change in reproduction, relative to control [b]
Control	-	82.7	28.1	-
A14111B	2500	68.5	15.8	17
	625	67.0	22.4	19
	312.5	88.0	31.4	-6

[a] The results for the test items treatments were individually compared to the control by one-way ANOVA. Treatment means did not differ significantly from the control ($P > 0.05$).

[b] A positive value indicates a decrease in reproduction and a negative value an increase in reproduction, relative to the control.

The validity criteria were met:

- Mortality within the control was ≤ 13% at 48 hr (i.e. 10%)
- Mortality within the toxic reference treatment was as expected in the lab (> 50% after 48 hr)(i.e. 60%)
- Fecundity in the control was ≥ 5 mummies/female and not more than 2 wasps produced 0 values.

Conclusion

Rates of 1250 – 5000 mL A14111B/ha had a significant effect on *Aphidius rhopalosiphi* survival, giving mortality of approximately 50%, though with no apparent rate-response. It was not possible to accurately calculate an LR_{50}, but the value can be stated as >625 mL A14111B/ha (corresponding to 262 g chlorothalonil/ha). None of the rates assessed, the maximum being 2500 mL, had any significant or >50% effect on reproductive performance. The ER_{50} is > 2500 mL A14111B/ha (corresponding to > 1048 g chlorothalonil /ha).

Report:	K-CP 10.3.2.1/02 Waterman L. (2004), Azoxystrobin and chlorothalonil: A rate-response laboratory test to determine the effects of a 80 + 400 g/L SC formulation (A14111B) on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-03-33, Mambo-Tox Ltd, Southampton, UK. (Syngenta File No. ICI5504/2181)
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. The LR50 is > 5000 mL A14111B/ha (corresponding to > 2095 g chlorothalonil /ha). The test item had statistically significant effects > 50% on the reproductive capacity of the exposed mites at rates between 1250 and 5000 mL A14111B/ha, but no clear dose response was evident.The ER50 was < 1250 mL/ha (corresponding to < 524 g chlorothalonil /ha)

Guidelines

Based on Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.

GLP: Yes.

Executive Summary

Azoxystrobin/chlorothalonil SC (80/400) is a suspension concentrate formulation (hereafter referred to as A14111B) nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil. The aim of the study was to determine the effects of dry residues of A14111B on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), under worst-case laboratory test conditions.

Following an initial range-finding test, A14111B was evaluated in a definitive test at five rates, equivalent to 5000, 2500, 1250, 625 and 312.5 mL product/ha. These treatments were compared to a control of deionised water and a toxic reference of BASF Perfekthion (nominally 400 g/L dimethoate) applied at a rate of 15 mL product/ha (nominally 6 g a.i./ha).

All treatments were applied to glass plates at a volume rate equivalent to 200 L spray solution/ha. The glass plates were left to dry and then placed onto damp tissue paper, with their treated surface uppermost. A ring of a sticky non-drying gel was drawn on each plate to create the arenas in which mites were then confined. Twenty protonymphal *T. pyri* were placed on each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was determined and they were then left *in situ* so that their reproduction could be assessed over a further 7 days. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

At 7 days, mortality in the control treatment was 11%, compared with 43%, 30%, 40%, 40%, and 20% in the 5000, 2500, 1250, 625 and 312.5 mL product/ha treatment rates of A14111B respectively. When adjusted for the control treatment deaths, the corrected mortality was 36%, 21%, 33%, 33% and 10% in the five respective treatment rates of A14111B. In the toxic reference treatment, 66% mortality (62% corrected) was recorded at 7 DAT.

Reproduction assessments were carried out for the highest three treatment rates of the test item. The mean number of eggs produced per female was 9.0 in the control treatment, compared with values of 0.8, 2.1 and 1.9 in the 5000, 2500 and 1250 mL/ha rates of A14111B respectively. The results for all of the test item treatments differed significantly from the control (ANOVA, $P < 0.001$).

It was concluded that there was no rate-response relationship with respect to mortality and that the 7-day LR_{50} (median lethal rate) was greater than the highest test rate (i.e. > 5000 mL A14111B/ha). The test item had statistically significant effects > 50% on the reproductive capacity of the exposed mites at rates between 1250 and 5000 mL A14111B/ha, but without a clear dose-response.

Materials	
Test Material:	azoxystrobin/chlorothalonil SC (80/400) (formulation code A14111B)
Description:	opaque cream-coloured suspension concentrate, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil
Lot/Batch #:	J7518/024
Purity:	80 g/L azoxystrobin (corresponding to 6.6%) and 419 g/L (corresponding to 34.6%) chlorothalonil
Stability of test compound:	Assumed stable pending re-analysis in September 2005
Vehicle and control:	Deionised water
Toxic reference:	Perfekthion EC (400 g dimethoate/L) in deionised water (15 mL product/ha)
Spray volume rate:	200 L spray solution/ha
Application method:	Potter Laboratory Spray Tower with static atomising nozzle, calibrated to deliver 200 L/ha.
Test organisms	
Species:	*Typhlodromus pyri* Sch. (Acari: Phytoseiidae)
Source:	Culture established at Test Facility in 1995.
Food:	Walnut and apple pollen.
Test substrate:	Glass.
Environmental test conditions	
Temperature:	25 to 27°C
Humidity:	51 to 84% relative humidity
Photoperiod:	16 h photoperiod (240-730 lux)

Study Design and Methods

Experimental dates: 6th January to 2nd February 2004.

The bioassay was initiated approximately 1 h after treatments had been applied to the glass test arenas, i.e. once residues had dried. The treated plates were placed onto damp tissue paper and a ring of a sticky non-drying gel drawn on each of them to create circular arenas in which mites were confined. Twenty protonymphal *T. pyri* of < 24 h old were placed at the centre of each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was determined and they were then left *in situ* so that their reproduction could be assessed over a further 7 days. These further assessments were carried out for the control and for the three highest treatment rates of the test item that had resulted in < 50% corrected mortality. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

Results and Discussion

The results of the mortality assessments are summarized in Table 10.3.2.1-3. At 7 days, mortality in the control treatment was 11%, compared with mortalities of 43%, 30%, 40%, 40% and 20% in the 5000, 2500, 1250, 625 and 312.5 mL/ha treatment rates of A14111B, respectively. When adjusted for the control treatment deaths, the corrected mortality was 36%, 21%, 33%, 33% and 10% in the five respective treatment rates of A14111B. In the toxic reference treatment, 66% mortality (62% corrected) was recorded at 7 DAT.

Table 10.3.2.1-3: Effects of fresh dry residues of A14111B on mortality of *the* mite *Typhlodromus pyri* when exposed under laboratory test conditions

Treatment	Rate (mL/ha)	Mean % mortality 7 DAT [a]	Corrected % mortality 7 DAT
Control	-	11	-
A14111B	5000	43**	36
	2500	30*	21
	1250	40**	33
	625	40**	33
	312.5	20	10
Perfekthion	15	66**	62

[a] The results for the mortality assessments were compared using Fisher's Exact Test. Asterisks indicate treatments that differed significantly from the control (* < P 0.01, ** P < 0.001).

The results of the reproduction assessments are summarized in Table 10.3.2.1-4. The mean number of eggs produced per female was 9.0 in the control treatment, compared with 0.8, 2.1 and 1.9 in the 5000, 2500 and 1250 mL/ha treatment rates of A14111B, respectively. The results for the three test item treatments differed significantly from the control (ANOVA, P < 0.001).

Table 10.3.2.1-4: Effects of residues of A14111B on reproduction of *Typhlodromus pyri* when exposed under laboratory test conditions

Treatment	Rate (mL/ha)	Mean number of eggs per female [a]	Effects on reproduction [b] (%)
Control	-	9.0	-
A14111B	5000	0.8*	91
	2500	2.1*	77
	1250	1.9*	79

[a] Treatments compared by one-way ANOVA. The test item treatments differed significantly from the control (* P < 0.001).

[b] Change in numbers of eggs per female, relative to control (after Blümel *et al*., 2000). A positive value indicates a decrease.

The validity criteria were met:

- Mortality in the control was ≤ 20% on day 7 after application (i.e. 11%)
- Mortality in the toxicity control was ≥ 50% at application rate of 9-15 mL formulated product/ha (i.e. 66%)
- Reproduction in the control was ≥ 4 eggs/females (i.e. 9)

Conclusion

No rate-response relationship was observed with respect to mortality and it was therefore concluded that the 7-day LR_{50} (median lethal rate) was greater than the highest test rate (i.e. > 5000 mL A14111B/ha or > 2095 g chlorothalonil /ha). There were statistically significant and >50% effects on fecundity at all rates assessed, from 1250 to 5000 mL/ha, although there was no apparent rate-response. The ER_{50} is < 1250 mL A14111B/ha (corresponding to < 524 g chlorothalonil /ha)

CP 10.1.1.1 Extended laboratory testing, aged residue studies with non-target arthropods

The following laboratory non-target arthropod studies, performed on A14111B, have not previously been reviewed and are provided in support of this assessment.

Report:	K-CP 10.3.2.2/01 Fussell S. (2004a) Azoxystrobin and chlorothalonil: A rate-response extended laboratory test to evaluate the effects of an 80 + 400 g/L SC formulation (A14111B) on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Report Number SYN-04-9, Mambo-Tox Ltd., Southampton, UK. (Syngenta File No. ICI5504/2395)
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. The LR_{50} and ER_{50} are > 5000 mL A14111B/ha (corresponding to > 2095 g chlorothalonil /ha).

Guidelines

Mead-Briggs *et al*. (in preparation). An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera, Braconidae).

GLP: Yes.

Executive Summary

Azoxystrobin/chlorothalonil SC (80/400) is a suspension concentrate (SC) formulation (hereafter referred to as A14111B) nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil. The aim of this study was to determine, under extended laboratory test conditions, the effects of A14111B on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae).

Following an initial range-finding test, A14111B was evaluated in a definitive test at three application rates, equivalent to 5000, 2500, 1250 mL A14111B/ha. Also included in the definitive test were a water-treated control and a toxic reference treatment of BASF Perfekthion (4 g dimethoate/ha). Treatments were applied to barley plants at a BBCH growth stage 12, trimmed to 10 cm height. Five female wasps <48 hours post emergence were introduced to each exposure test unit. Six replicate exposure units were established for each treatment and control. The condition of the wasps was assessed 2, 24 and 48 hours after they were introduced to the test units. Thirty minutes after introduction of the wasps, observations for potential repellence were started which were repeated every 30 minutes for the first three hours of exposure. For the subsequent reproduction assessment, the performance of 15 individually-confined female wasps was evaluated per treatment.

Treatment with A14111B did not result in any repellent effect with *A. rhopalosiphi* during the initial three-hour observation period. No mortalities were observed in the control or in any of the A14111B test treatments during the 48-hour observation period. No significant effect on the reproductive performance of *A. rhopalosiphi* was observed following exposure to A14111B.

The 48-h LR_{50} was determined to be >5000 mL A14111B/ha (the highest rate tested). There were no effects >50% on fecundity at any rate tested, up to and including 5000 mL A14111B/ha.

Materials

Test Material:	Azoxystrobin/chlorothalonil SC (80/400) (formulation code A14111B)
Description:	Opaque cream-coloured suspension concentrate, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil
Lot/Batch #:	J7518/024
Purity:	80 g/L azoxystrobin (corresponding to 6.6%) and 419 g/L chlorothalonil (corresponding to 34.6%)
Stability of test compound:	Assumed stable pending re-analysis in September 2005
Vehicle and control:	Deionised water
Toxic reference:	Perfekthion (nominal 400 g dimethoate/L) in deionised water (4 g dimethoate/ha)
Spray volume rate:	400 L spray solution/ha
Application method:	Potter Laboratory Spray Tower
Test organisms	
Species:	*Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae)
Source:	Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*).
Food:	10% w/v fructose solution sprayed on plants
Test substrate:	Barley plants at a BBCH growth stage 12
Environmental test conditions	
Temperature:	19-22°C
Humidity:	67-84%
Photoperiod:	16 hour daily photoperiod

Study Design and Methods

Experimental dates: 14th April to 5th July 2004.

The effect of fresh residues of the test substance, applied to barley leaves, on the mortality and subsequent reproduction of *A. rhopalosiphi* was compared to an untreated deionised water control and a toxic reference (4 g dimethoate/ha). Based on an initial range finding test, A14111B was applied at rates equivalent to 1250, 2500 and 5000 mL/ha.

Exposure phase: Groups of 10 barley seedlings (*Hordeum vulgare*) where grown in shallow 11 cm diameter pots until BBCH growth stage 12 (~10 days after sowing) and were trimmed to an even height of 10 cm. Approximately 60-90 minutes before treatment application the plants were sprayed with a 10% w/v fructose solution to provide both food and a foraging stimulus for the subsequently

introduced wasps. The soil in the pots was covered with silver sand to create a uniform surface before the treatment application. The test treatments were applied in a spray volume equivalent to 400 L/ha using a modified Potter Laboratory Spray Tower. The treated plants were left to dry on a laboratory bench before being enclosed within a clear acrylic cylinder (8-9 cm diameter, 20cm high), the top of which was covered with nylon netting. Five female wasps <48 hours post emergence were introduced to each exposure test unit. Six replicate exposure units were established for each treatment and control. The condition of the wasps was assessed 2, 24 and 48 hours after they were introduced to the test units. Thirty minutes after introduction of the wasps, observations for potential repellence were started, which were repeated every 30 minutes for the first three hours of exposure. Environmental conditions were monitored continually throughout the exposure period.

Reproduction phase: 48 hours after introduction of the wasps to the exposure test units, fifteen wasps from the control and each treatment in which corrected mortality was <50%, were transferred to pots containing 10-20 untreated barley seedlings which had been infested six days previously with host aphids (>100 adults and nymphs of *Metopolophium dirhodum* and *Rhopalosiphum padi*). The barley plants were enclosed within a test unit composed of a clear acrylic cylinder 9 cm diameter, 20 cm high, the top of which was sealed with nylon mesh. A single wasp was introduced into each reproduction test unit; 15 test units were established for each treatment and control. The adult female wasps were removed after 24 hours. The number of mummies that developed was recorded after a further 11 days. Environmental conditions were monitored continually throughout the exposure period.

Results and Discussion

The results of the effects of A14111B on mortality and reproduction of *A. rhopalosiphi* are shown in the table below.

Table 10.3.2.2-1: Effects of residues of A14111B on the mortality and reproduction of *A. rhopalosiphi* under extended laboratory conditions

Treatment (mL A14111B/ha)	48-hour Mortality (%)	Mean number of mummies per surviving female	% reduction in reproduction relative to control
Control (0)	0	77.9 ± 28.4	-
1250	0	64.5 ± 41.9	17
2500	0	57.1 ± 17.8	27
5000	0	70.9 ± 25.0	9
Toxic reference	93	-	-

Treatment with A14111B did not result in any repellent effect with *A. rhopalosiphi* during the initial three-hour observation period. No mortalities were observed in the control or in any of the A14111B

test treatments during the 48-hour observation period. No significant effect on the reproductive performance of *A. rhopalosiphi* was observed following exposure to A14111B.
The validity criteria were met:

- Mortality within the control was ≤ 13% at 48 hr (i.e. 0%)
- Mortality within the toxic reference treatment was as expected in the lab (> 50% after 48 hr)(i.e. 93%)
- Fecundity in the control was ≥ 5 mummies/female and not more than 2 wasps produced 0 values.

Conclusion

The 48-h LR_{50} was determined to be >5000 mL A14111B/ha (corresponding to > 2095 g chlorothalonil /ha) . There were no effects >50% on fecundity at any rate tested, up to and including 5000 mL A14111B/ha.

Report:	K-CP 10.3.2.2/02 Waterman L. (2004a). Azoxystrobin and chlorothalonil: A rate-response extended laboratory test to determine the effects of an 80 + 400 g/L SC formulation (A14111B) on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-04-8. Mambo-Tox Ltd, Southampton, UK. (Syngenta file No. ICI5504/2381)
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. A deviation from the test guideline was the number of replicates (4 instead of 5). This is however not considered to invalidate the study. The LR_{50} is > 5000 mL A14111B/ha (corresponding to > 2095 g chlorothalonil/ha). The ER_{50} was calculated by RMS using Probit analysis, and was 2833 mL product/ha (1187 g chlorothalonil/ha).

Guidelines

Blümel *et al*. (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.

GLP: Yes.

Executive Summary

Azoxystrobin/chlorothalonil SC (80/400), hereafter referred to as A14111B, is a suspension concentrate (SC) formulation nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil.
The aim of the study was to determine the effects of fresh dry residues of A14111B on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), under extended laboratory test conditions.

Following an initial range-finding test, A14111B was evaluated in a definitive test at four rates, equivalent to 5000, 1000, 200 and 40 mL product/ha. These variants were compared to a control treatment of deionised water and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate) applied at a rate of 30 mL product/ha (nominally 12 g a.i./ha). All treatments were applied to leaf discs taken from French bean plants (*Phaseolus vulgaris* L.), at a volume rate equivalent to 200 L spray solution/ha. The leaf discs were left to dry and then placed onto wet cotton wool, with their treated surface uppermost. A ring of a sticky non-drying gel was drawn on each disc to create the arenas in which mites were then confined. Twenty protonymphal *T. pyri* were placed on each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was then determined and they were left *in situ* so that their reproduction could be assessed over a further 7 days. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated. These reproduction assessments were made for mites from all treatment rates of the test item that had resulted in < 50% corrected mortality, and from the control treatment.

At 7 DAT, mortality in the control treatment was 8%, compared to 19%, 8%, 4% and 6% in the 5000, 1000, 200 and 40 mL product/ha treatment rates of A14111B, respectively. When adjusted for the control treatment deaths, the corrected mortalities were 12%, 0%, 0% and 0% in the four respective treatment rates of A14111B. In the toxic reference treatment, 96% mortality (96% corrected) was recorded at 7 DAT.

In the reproduction assessments, the mean number of eggs produced per female was 9.8 in the control, compared with values of 4.1, 6.3, 8.9 and 9.9 in the 5000, 1000, 200 and 40 mL/ha treatment rates of A14111B, respectively. The results for the 5000 and 1000 mL product/ha treatment rates differed significantly from the control (ANOVA, $P < 0.001$ and $P < 0.01$, respectively), but the results for the 200 and 40 mL/ha treatment rates did not differ significantly from the control ($P > 0.05$).

In conclusion, no rate-response relationship was observed with respect to A14111B and mite mortality and it was therefore concluded that the 7-day LR_{50} (median lethal rate) was greater than the highest test rate of 5000 mL/ha. A14111B had no significant effect on the reproduction of mites at rates of up to and including 200 mL product/ha.

Materials

Test Material:	Azoxystrobin/chlorothalonil SC (80/400) (formulation code A14111B)
Description:	Opaque cream-coloured liquid, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil
Lot/Batch #:	J7518/024
Purity:	80 g/L azoxystrobin (corresponding to 6.6%) and 419 g/L chlorothalonil (corresponding to 34.6%)
Stability of test compound:	Assumed stable pending re-analysis in September 2005

Vehicle and control:	Deionised water
Toxic reference:	Perfekthion EC (nominal 400 g dimethoate/L) in deionised water (30 mL product/ha)
Spray volume rate:	200 L spray solution/ha
Application method:	Potter Laboratory Spray Tower, calibrated for each treatment preparation.
Test organisms	
Species:	*Typhlodromus pyri* (Acari: Phytoseiidae).
Source:	Culture maintained at Test Facility.
Food:	1:1 v/v mixture of walnut (*Juglans regia* L.) and apple (*Malus* sp. var. Winter Banana)
Test substrate:	Leaf discs taken from first true leaves of dwarf French beans (*Phaseolus vulgaris* L., var. The prince).
Environmental test conditions	
Temperature:	24 to 30°C
Humidity:	69 to 96% relative humidity
Photoperiod:	16 h photoperiod (390-762 lux)

Study Design and Methods

Experimental dates: 27th April to 29th June 2004.

The test substrate comprised leaf discs taken from dwarf French bean plants, *Phaseolus vulgaris.* The bioassay was initiated approximately 1 h after treatments were applied, i.e. once residues on the leaf discs had dried. The leaf discs were placed onto damp cotton wool and a ring of a sticky non-drying gel drawn around the edge of each to create circular arenas in which mites were confined. Twenty protonymphal *T. pyri* were placed at the centre of each replicate arena, with four replicates (80 mites in total) prepared per treatment. The mites were fed regularly with untreated pollen for food. Their survival was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was determined and they were then left *in situ* so that their reproduction could be assessed over a further 7 days. These further assessments were carried out for the control and for treatment rates of the test item that had resulted in < 50% corrected mortality. The mean number of eggs produced per female between 7 and 14 days after treatment (DAT) was calculated.

The ER_{50} was not included in the report and was therefore calculated by the RMS using Probit analysis.

Results and Discussion

The results of the mortality assessments are summarised in Table 10.3.2.2-2.

Table 10.3.2.2-2: Effects of residues of A14111B on mortality of the mite *Typhlodromus pyri* under extended laboratory test conditions

Treatment	Rate (mL/ha)	Mean % mortality 7 DAT [a]	Corrected % mortality 7 DAT
Control	-	8	-
A14111B	5000	19	12
	1000	8	0
	200	4	0
	40	6	0
Perfekthion	30	96*	96

[a] The results for the mortality assessments were compared using Fisher's Exact Test. Asterisks indicate treatment means that differed significantly from the control (*P < 0.001).

The results of the reproduction assessments are summarized in Table 10.3.2.2-3.

Table 10.3.2.2-3: Effects of residues of A14111B on reproduction of the mite, *Typhlodromus pyri*, under extended laboratory test conditions

Treatment	Rate (mL/ha)	Mean number of eggs per female [a]	Effects on reproduction [b] (%)
Control	-	9.8	-
A14111B	5000	4.1**	58
	1000	6.3*	36
	200	8.9	9
	40	9.9	-1

[a] Treatments compared by one-way ANOVA. Asterisks indicate test item treatments that differed significantly from the control (* P < 0.01, ** P < 0.001).

[b] Change in numbers of eggs per female, relative to control (after Blümel *et al*., 2000). A positive value indicates a decrease.

The validity criteria were met:

- Mortality in the control was ≤ 20% on day 7 after application (i.e. 8%)
- Mortality in the toxicity control was as expected (i.e. 96%)
- Reproduction in the control was ≥ 4 eggs/females (i.e. 9.8)

Conclusion

No rate-response relationship was observed with respect to mortality and it was therefore concluded that the 7-day LR_{50} (median lethal rate) was greater than the highest test rate of 5000 mL A14111B/ha (>2095 g chlorothalonil/ha). A14111B had no significant effect on the reproduction of mites at rates of up to and including 200 mL product/ha. However, fecundity in the 1000 and 5000 mL/ha A14111B treatments was statistically significantly reduced when compared to the untreated control, and in the 5000 mL/ha treatment effects were > 50%. The ER_{50}, calculated by RMS, was 2833 mL/ha, which is equivalent to 1187 g chlorothalonil/ha.

Report:	K-CP 10.3.2.2/03 Douglas B. (2004). Azoxystrobin and chlorothalonil: A rate-response extended laboratory test to evaluate the effects of an 80 + 400 g/L SC formulation (A14111B) on the green lacewing, *Chrysoperla carnea* (Neuroptera, Chrysopidae). Report Number SYN-04-10. Mambo-Tox Ltd, Southampton, UK. (Syngenta file No. ICI5504/2486)
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. The LR_{50} was greater than the highest test rate, i.e. > 5000 mL A14111B/ha (corresponding to > 2095 g chlorothalonil/ha). The ER_{50} was > 5000 mL A14111B/ha (corresponding to > 2095 g chlorothalonil /ha).

Guidelines

Vogt *et al*. (2000). Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae).

GLP: Yes.

Executive Summary

Azoxystrobin/chlorothalonil SC (80/400), hereafter referred to as A14111B, is a suspension concentrate formulation nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil. The aim of the study was to evaluate the effects of A14111B on the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae), under extended laboratory test conditions. The reproductive potential of the resultant adult lacewings was also checked.

A14111B was evaluated at five application rates, equivalent to 5000, 2500, 1000, 200 and 40 mL product/ha. These were compared to a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 150 mL/ha (nominally 60 g a.i./ha). Treatments were applied to leaves of the dwarf French bean (*Phaseolus vulgaris* L.) and, once residues had dried, the leaves were used to line the floor of test arenas (n = 40 per treatment) into which individual larvae of *C. carnea* (2-3 days old) were introduced. The larvae were fed with untreated eggs of the Angoumois grain moth, *Sitotroga cerealella* (Oliver) and any pre-imaginal

mortality of the lacewings was recorded. A check was then made for sub-lethal effects on the reproductive performance of the adults surviving in the control and in the three highest treatment rates of the test item. For this, the egg-laying activity of grouped females was monitored for two 24-h periods and the viability of the eggs was determined.

Pre-imaginal mortality in the control treatment was 15%, compared with 21%, 28%, 31%, 23% and 28% in the 5000, 2500, 1000, 200 and 40 mL/ha treatment rates of A14111B, respectively, and 93% in the toxic reference treatment. The corrected mortalities were therefore 7%, 15%, 19%, 10% and 16% in the respective test item treatments and 91% in the toxic reference. Statistically, the mortality in the 5000, 2500, 1000, 200 and 40 mL/ha treatment rates did not differ significantly from the control ($P > 0.05$).

The mean number of eggs produced per female per day was 28 in the control, compared with values of 32, 27 and 26 in the 5000, 2500 and 1000 mL/ha treatment rates of A14111B. The mean percentage egg viability was 88% in the control and 89%, 86% and 86% in the respective test item treatments. Since the mean numbers of eggs produced in all test item treatments was ≥ 15 eggs/female/day and the mean egg viability was ≥ 70%, this was indicative of there being no harmful treatment effects on lacewing reproduction (Vogt *et al*., 2000).

In conclusion, no rate-response relationship was determined for the effects of A14111B on the lacewing, *Chrysoperla carnea*, under extended laboratory test conditions. The LR_{50} was therefore taken to be greater than the highest test rate, i.e. >5000 mL A14111B/ha (corresponding to > 2095 g chlorothalonil /ha). In addition, no effect on reproduction was observed at rates up to and including 5000 mL A14111B/ha (corresponding to > 2095 g chlorothalonil /ha).

Materials

Test Material:	Azoxystrobin/chlorothalonil SC (80/400) (formulation code A14111B)
Description:	Opaque cream-coloured liquid, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil
Lot/Batch #:	J7518/024
Purity:	80 g/L azoxystrobin (corresponding to 6.6%) and 419 g/L chlorothalonil (corresponding to 34.6%)
Stability of test compound:	Assumed stable pending re-analysis in September 2005
Vehicle and control:	Deionised water
Toxic reference:	Perfekthion EC (400 g dimethoate/L) in deionised water (150 mL product/ha)
Spray volume rate:	200 L spray solution/ha
Application method:	Modified Potter Laboratory Spray Tower, calibrated for each treatment preparation.
Test organisms	
Species:	*Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae).

Source:	Culture maintained at Test Facility.
Food:	UV-killed eggs of the Angoumois grain moth, *Sitotroga cerealella* (Oliver) (Lepidoptera, Gelechiidae)
Test substrate:	Leaf discs taken from first true leaves of dwarf French beans (*Phaseolus vulgaris* L., var. The prince).
Environmental test conditions	
Temperature:	21 to 27°C
Humidity:	52 to 95% relative humidity
Photoperiod:	16 h photoperiod (2630-4210 lux)

Study Design and Methods

Experimental dates: 1st July to 12th August 2004.

Excised French bean leaves (40 replicates per treatment) were treated by spraying (181-219 L/ha, corresponding to 92% and 110% of the target rate) on their upper (adaxial) surface and left for up to 1 h to dry. Arenas were then assembled and 2- to 3-day-old lacewing larvae individually confined on the upper treated surface. The larvae were provided with untreated moth eggs for food and pre-imaginal mortality was assessed. The adults were then grouped together, with treatments kept in separate boxes. A check was made for sub-lethal effects on the reproductive performance of the surviving adults in the control and in the highest three treatment rates of the test item. For this the egg-laying activity of all surviving females was monitored for two 24-h periods in one week and the viability of the eggs produced was then determined.

Results and Discussion

The results of the mortality assessments are summarised in Table 10.3.2.2-4.

Table 10.3.2.2-4: Effects of residues of A14111B on mortality of the lacewing, *Chrysoperla carnea,* exposed under extended laboratory test conditions

Treatment	Rate (mL/ha)	% pre-imaginal mortality at 48 h [a]	Corrected % pre-imaginal mortality [b]
Control		15	-
A14111B	5000	21	7
	2500	28	15
	1000	31	19
	200	23	10
	40	28	16
Perfekthion	150	93*	91

[a] Data from individual treatments were compared to the control using Fisher's Exact Test (α = 0.05). Mortality in treatments marked with asterisks differed significantly from the control (* P < 0.001).

[b] The corrected pre-imaginal mortality was calculated using Abbott's formula (Abbott, 1925).

The results of the reproduction assessments are summarised in Table 10.3.2.2-5.

Table 10.3.2.2-5: Effects of residues of A14111B on the reproductive capacity of the lacewing, *Chrysoperla carnea,* exposed under extended laboratory test conditions

Treatment	Rate (mL/ha)	Mean number eggs/female/day [a]	Mean percentage viability [b]
Control	-	28	88
A14111B	5000	32	89
	2500	27	86
	1000	26	86

[a] Based on two 24-h-long assessments made for each oviposition box in each treatment.

[b] Based on all eggs laid on the fibrous tissue sheet lining the lid of each oviposition box

No statistically significant effects on reproduction were observed; the mean number of eggs produced/female/day was ≥ 15 and the mean egg viability was ≥70 % in all the test treatments. These thresholds are currently viewed as being indicative of no harmful effects, according to the test guideline.

The validity criteria were met:

- Cumulative mortality in the control was ≤ 20% (i.e. 15%)
- Fecundity in the control was ≥ 15 eggs per female per day (i.e. 28)

- Fertility in the control was ≥ 70% mean hatching rate (i.e. 88%)
- Level of mortality in the reference treatment was ≥ 50% (i.e. 91%).

Conclusion

When exposed to dried residues of A14111B/ha on bean leaves, the LR_{50} for *C. carnea* was determined to be >5000 mL/ha (corresponding to > 2095 g chlorothalonil/ha). There were no effects on reproduction at any tested rate, up to and including 5000 mL/ha, the highest rate tested. The ER_{50} was > 5000 mL/ha (corresponding to > 2095 g chlorothalonil /ha).

B.9.6 Risk assessment for arthropods

B.9.6.1 Risk assessment for bees

Table B.9.6.1-01: Table of toxicity data for bees

Organism	Test item	Test type	EU endpoint[a]	Endpoint used in the risk assessment
Apis mellifera	Chlorothalonil	48h oral	LD_{50} >40 µg/bee	LD_{50} **>40** µg/bee
		48h contact	LD_{50} >63 µg/bee	LD_{50} **>63** µg/bee
		Adult Chronic		10 d NOED = 188 mg a.s./kg diet; ca. 6.5 µg a.s./bee/day LDD_{50} = **53.9 µg a.s./bee/day**.
		Larval development	-	7 d NOED = 91 mg a.s./kg diet (14.5 µg total a.s./larva = 3.6 µg a.s./larva/day LD_{10} = 10 µg total a.s./larva **= 2.5 µg a.s./larva/day**
	A14111B	48h oral	-	LD_{50} = **>917** µg/bee (> 317 µg chlorothalonil/bee)
		48h contact	-	LD_{50} **>1531** µg/bee (> 523 µg chlorothalonil/bee)
		Adult Chronic	-	NOEC = 606 mg/kg food; NOED = 29.1 µg/bee/day LDD_{50} = **171** µg prod/bee/day
		Larval development	-	8 d NOEC = 198 mg/kg diet NOED =31.3 µg total prod/larva = **7.8 µg prod/larva/day**
Bombus terrestris	Chlorothalonil	96 h oral		LD_{50} **>94** µg/bee
		96 h contact		LD_{50} **>100** µg/bee

~~**Exposure**~~

~~Applications of pesticides can potentially result in exposure of bees either through direct over-spray, or by contact with residues on plants whilst bees are foraging for food.~~

The risk to bees has been assessed following the EPPO 2010 scheme[18] as proposed in the list of guidance documents relevant to the implementation of Regulation 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

B.9.6.1.1 Acute risk assessment

The potential acute risk from use of A14111B was assessed using the maximum single application rates and the LD_{50} values to calculate hazard quotients in accordance with the current Terrestrial Guidance Document[19] and EPPO 2010.

Table 10.3.1-2: Risk to bees from oral exposure to A14111B and chlorothalonil

Crop	Test substance	Application rate (g/ha)	Species	Oral LD_{50} (µg/bee)	Hazard quotient
Cereals	A14111B	2286 [a]	*Apis mellifera*	>917	<2.5
	Chlorothalonil	750		>40	<19
Tomatoes	A14111B	3048 [b]	*Apis mellifera*	>917	<3.3
	Chlorothalonil	1000		>40	<25

[a] A14111B applied at 1.875 L/ha; density 1.219 g/cm^3

[b] A14111B applied at 2.5 L/ha; density 1.219 g/cm^3

All the hazard quotients for chlorothalonil and A14111B are less than 50, indicating that the risk to bees is acceptable following use of A14111B according to the proposed use pattern. The contact LD_{50} for bumble bee does not indicate that bumble bee is acutely more sensitive than honey bee.

Table 10.3.1-3: Risk to bees from contact exposure to A14111B and chlorothalonil

Crop	Test substance	Application rate (g/ha)	Species	Contact LD_{50} (µg/bee)	Hazard quotient
Cereals	A14111B	2286 [a]	*Apis mellifera*	>1531	<1.5
	Chlorothalonil	750		>63	<12
Tomatoes	A14111B	3048 [b]	*Apis mellifera*	>1531	<2.0
	Chlorothalonil	1000		>63	<16

[a] A14111B applied at 1.875 L/ha; density 1.219 g/cm^3

[b] A14111B applied at 2.5 L/ha; density 1.219 g/cm^3

[18] EPPO/OEPP (2010) Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(3)). Bulletin OEPP/EPPO Bulletin 40: 323-331.

[19] Anonymous (2002b). Guidance Document on terrestrial ecotoxicology under Council Directive 91/414/EEC. SANCO/10329/2002. 17 October 2002.

All the hazard quotients for chlorothalonil and A14111B are less than 50, indicating that the risk to bees is acceptable following use of A14111B according to the proposed use pattern. The oral LD_{50} for bumble bee does not indicate that bumble bee is acutely more sensitive than honey bee.

B.9.6.1.2 Chronic Risk Assessment

Chronic adult and larval bee studies have been conducted according to the data requirements under 1007/2009. The endpoints from these studies have been assessed by adapting the EPPO 2010 scheme.

Larval assessment

Following the EPPO scheme for assessing potential risks to larvae (point 4 on the scheme), the scheme suggests that effects on growth or development can be excluded when considering chlorothalonil, since it is not an IGR, and shows no effects on juvenile stages in other organisms as demonstrated by the risk assessments for non-target arthropods, and soil organisms (Collembola and *Hypoaspis*). Thus chlorothalonil can be categorised as posing a low risk to bees.

However a chronic larval study is available and this potential low risk can be further demonstrated by carrying out a worst-case risk assessment through the calculation of a TER value as set out in the EPPO 2010 scheme (point 5 on the scheme).

A worst-case of potential exposure via residues in pollen / nectar can be estimated based on the default worst-case residue of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6), based on a database of measured values from aerial plant parts as a surrogate for nectar and pollen.
The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst case data from **Rortais *et al.*, 2005**[20] as proposed in the EPPO scheme have been used to estimate the consumption by bee larvae:

Worst case: drone larvae consuming 98.2 mg sugar in 6.5 days (= 15.1 mg sugar /day)[21].
Thus considering residues of 1 mg a.s./kg nectar x consumption of 15.1 mg sugar/bee/day (100 mg nectar/bee/day)

Total exposure ETE = 0.1 µg a.s./bee/day

This can be compared to the chlorothalonil larval NOEC of 10.3 µg a.s./bee/developmental period, which is = 2.6 µg a.s./bee/day.

- TER = NOEL (µg a.s./bee/day)/ETE (µg a.s./larva/day)

= 2.6/0.1 = 26

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees, thus it I considered likely that the proposed uses of chlorothalonil pose an acceptable risk to bee larval

[20] Agnès RORTAIS, Gérard ARNOLD, Marie-Pierre HALM, Frédérique TOUFFET-BRIENS (2005) Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36 (2005) 71–83

development. Nonetheless, it is noted that from the study of Zhu et. al. (2014), a lower LC_{50} was found (< 5.44 µg a.s./bee). Since this value is even lower than the NOEC used above, the RMS must consider (1) the reliability of the endpoints and (2) the conservativeness of the risk assessment, overall. The dossier study was considered fully reliable, and the study from the public literature was also considered well performed, though several drawbacks were noted, including the fact that (1) standard Guidelines were not followed, (2) only a limit, nominal concentration was used, (3) there was no positive control, (4) mortality in the blank and solvent controls (pooled) was > 15% (17.2%). Further, the test was performed using chlorothalonil, whereas the dossier test was performed using the formulation. Zhu et. al. (2014) used acetone and methanol (1%) to dissolve the technical a.s. into the larval diet, and included two solvent controls and an untreated control, which did not show significantly different levels of larval mortality. Thus, it seems unlikely that the presence of solvent has resulted in the increased toxicity to larvae and it can be concluded that the formulation is therefore of lower toxicity. Since the test with the formulation was fully acceptable, the risk to honey bee larvae from A14111B is considered addressed by the formulation study.

Adult chronic assessment

The notifier performed the adult chronic risk assessment according to the EPPO 2010 scheme, which does not recommend a chronic assessment for adults for foliar spray applications. However, as an approach is proposed as an assessment refinement for seed coatings/soil treatments (point 7 on the scheme), the notifier adapted this approach to provide an assessment for foliar sprays.

A worst-case of potential exposure via residues in pollen / nectar can be estimated as before based on the default worst-case value of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6), based on a database of measured values from aerial plant parts as a surrogate for nectar and pollen.
The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst case data from Rortais *et al.*, 2005 as proposed in the EPPO 2010 scheme, have been used to estimate the consumption by bee foragers:
Worst case: forager consuming 128 mg nectar/day.
Thus considering residues of 1 mg a.s./kg nectar x consumption of 850 mg nectar/bee/day (see footnote 21)

Total exposure ETE = 0.85 µg a.s./bee/day

This can be compared to the chlorothalonil adult NOEL of 9.2 µg a.s./bee/day.

- TER = NOEL (µg a.s./bee/day)/ ETE (µg a.s./bee/day)

= (9.2/0.85) = 10.8

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees when a NOEL is used in this assessment, thus, it is considered that the proposed uses of chlorothalonil pose an acceptable chronic risk to adult bees.

[21] It is noted that the EFSA (2014) guidance uses 15% of sugar content in nectar for crops, thus the risk assessment was updated to include this conservative assumption for nectar consumption.

~~Tests on chronic toxicity and larval and brood development have been carried out in accordance with the Annexes to Regulation 283/2013 and 284/2013. The results of these tests indicate that the use of chlorothalonil in A14111B poses an acceptable risk to bees.~~

~~Metabolites~~

~~The notifier has not discussed the relevance of or potential risk from the metabolites of chlorothalonil, particularly the relevant plant metabolite SDS-3701, and states that this is less relevant considering the low toxicity of the parent and probable low exposure. The RMS does not agree that the toxicity of the parent is predictive of the toxicity of the relevant plant metabolite SDS-3701, which is significantly more toxic to birds and mammals. Further, the toxicity of the parent to larvae and chronically to adult bees is in question, as the available tests were performed with formulations and the public literature data suggests higher toxicity to lavae from the technical active substance.~~

Other bee species

The chronic and larval risk assessments above are performed for honey bee, and could not be finalized (larval). There is no information on effects on bumble bee or solitary bee species. One study in the public literature (see CA 9.1.3.5 for more information) suggested effects on bumble bees at the colony level, however, a number of short-comings of the study make it insufficient for purposes of regulatory risk assessment. Based on the acute risk assessment for bumble bees provided above, it can at least be concluded that the acute risk to bumble bees from the proposed uses is acceptable.

Consideration of public literature

The notifier eliminated much of the public literature on chlorothalonil as irrelevant to the risk assessment, as it focusses on sublethal or indirect effects and does not provide quantitative endpoints for use in risk assessment. Nonetheless, the RMS finds that several of these studies are relevant and should be submitted for consideration. See CA 9.1.3.5 for more information.

Risk assessment for bees (EFSA 2014[22])

During the EU review, EFSA and the RMS requested a risk assessment for honeybees according to the latest EFSA risk assessment guidance document. The Notifier considers that risk assessment to this guidance is not appropriate for regulatory decision making at EU level, as the guidance is not agreed by all member states and as such has not been noted. However, given the direct request of EFSA, a partial assessment has been provided by the notifier, and checked by the RMS. The notifier did not present a risk assessment for parts of the Guidance where they determined that significant uncertainty reamained (e.g. water exposure, HPG assessment and bumble and solitary bee assessments). The RMS has included an assessment of bumble bees for those areas of the risk assessment where data was available.

[22] European Food Safety Authority, 2013 (***updated 04 July 2014***). EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295

The risk assessment guidance is structured in a stepwise manner beginning with a screening step assessment, those scenarios which pass the screening step are considered to demonstrate acceptable risk and as such will not be considered at higher tiers of assessment. At higher tiers treated crop, weeds, field margins and next crop are all addressed.

All calculations were performed using the EFSA Bee calculator Tool (Bee-Tool v.3) available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3295/full.

Table 10.3.1-4: Crop groupings and critical use patterns relevant to the use of A14111B

Test substance	GAP crop species	Application category	Critical use pattern		
			Rate (kg/ha)	No. of apps	App. Interval (days
Chlorothalonil	Cereal BBCH 30-69	Downward spray	0.75	2	14
	Tomatoes BBCH 51-89	Downward spray	1.0	1	-
A14111B	Cereal BBCH 30-69	Downward spray	2.286	2	14
	Tomatoes BBCH 51-89	Downward spray	3.048	1	-

Screening Step

Acute contact assessment

The acute contact screening step assessment is calculated by dividing the application rate in g a.s./ha by the acute contact LD_{50} value (µg a.s./bee). The subsequent **$HQ_{contact}$** is considered to demonstrate acceptable risk where it is less than the applicable trigger value.

$HQ_{contact}$ = application rate (g a.s./ha) / $LD_{50\ contact}$ (µg a.s./bee)

$HQ_{contact}$ values for acute exposure to chlorothalonil following use of A14111B according to the proposed uses are given in the table below.

Table 10.3.1-5: Screening step – Risk assessment of acute contact exposure to chlorothalonil

Test substance	Crop Group	Species	App. rate (g a.s./ha)	LD_{50} contact (µg/bee)	$HQ_{contact}$	Trigger
Chlorothalonil	Cereals	Honeybee	750	>101	<7.4	42
	Cereals	Bumble bee	750	> 100	**<7.5**	7
	Tomatoes	Honeybee	1000	>101	<9.9	42
	Tomatoes	Bumble bee	1000	> 100	**<10.0**	7
A14111B	Cereals	Honeybee	2286	>1531	<1.50	42
	Tomatoes	Honeybee	3048	>1531	<2.0	42

HQ/ETRs in **bold** are above the relevant trigger and require further refinementHQ/ETRs in **bold** are above the relevant trigger and require further refinement

The **$HQ_{contact}$** value for chlorothlaonil is less than the trigger of 42 for honeybees, according to EFSA 2014, indicating that acute risk to honeybees is acceptable following use of A14111B according to the proposed use pattern. The **$HQ_{contact}$** value for chlorothlaonil is potentially than the trigger of 7 for bumble bees, according to EFSA 2014, indicating that acute risk to bumble bees is acceptable following use of A14111B according to the proposed use pattern cannot be exluded and a Tier 1 assessment for acute contact risk to bumble bees should be performed.

Acute oral assessment

The acute oral screening step assessment is calculated by multiplying the application rate in kg a.s./ha by a shortcut value as defined in the guidance. The resulting exposure value is then divided by the acute oral LD_{50} value (µg a.s./bee). The subsequent **$ETR_{acute\ adult\ oral}$** is considered to demonstrate acceptable risk where it is less than the applicable trigger value.

$ETR_{acute\ adult\ oral}$ = application rate (kg a.s./ha) x SV / $LD_{50\ oral}$ (µg a.s./bee)

$ETR_{acute\ adult\ oral}$ values for acute exposure to chlorothalonil following use of A14111B according to the proposed uses are given in the table below.

Table 10.3.1-6: Screening step – Risk assessment of acute oral exposure to chlorothalonil

Test substance	Crop Group	Species	App. rate (g a.s./ha)	Calculatiuon factor (Ef x SV)	LD_{50} oral (µg./bee)	ETR	Trigger
Chlorothalonil	Cereals	Honeybee	750	7.6	>63	<0.09	0.2
	Cereals	Bumble bee	750	11.2	>94	<0.09	0.036
	Tomatoes	Honeybee	1000	7.6	>63	<0.12	0.2
	Tomatoes	Bumble bee	1000	11.2	>94	<0.12	0.036
A14111B	Cereals	Honeybee	2286	7.6	>917	<0.02	0.2
	Tomatoes	Honeybee	3048	11.2	>917	<0.03	0.2

HQ/ETRs in **bold** are above the relevant trigger and require further refinementHQ/ETRs in **bold** are above the relevant trigger and require further refinement

The **$ETR_{acute\ adult\ oral}$** value for chlorothalonil and A14111B are below the trigger of 0.2 for downward sprays, indicating that acceptable acute risk to honeybees. The **$ETR_{acute\ adult\ oral}$** value for chlorothalonil is potentially above the trigger of 0.036 for downward sprays, indicating that an acute risk to bumble bees cannot be excluded. A tier 1 risk assessment should be performed.

Chronic adult oral assessment

The chronic adult oral screening step assessment is calculated by multiplying the application rate in kg a.s./ha by a shortcut value as defined in the guidance. The resulting exposure value is then divided by the chronic (10 day) adult oral LDD_{50} value (µg a.s./bee/day). The subsequent **$ETR_{chronic\ adult\ oral}$** is considered to demonstrate acceptable risk where it is less than the applicable trigger value.

$ETR_{chronic\ adult\ oral}$ = application rate (kg a.s./ha) x SV / $LDD_{50\ oral}$ (µg a.s./bee/day)

$ETR_{chronic\ adult\ oral}$ values for chronic exposure to chlorothalonil following use of A14111B according to the proposed uses are given in the table below.

Table 10.3.1-7: Screening step – Risk assessment of chronic oral exposure to chlorothalonil

Test substance	Crop Group	Species	App. rate (kg a.s./ha)	Shortcut Value (downward spray)	LD_{50} oral (µg./bee/day)	$ETR_{chronic\ adult\ oral}$	Trigger
Chlorothalonil	Cereals	Honeybee	0.75	7.6	53.9	**0.106**	0.03
	Tomatoes		1.0	7.6	53.9	**0.141**	0.03
A14111B	Cereals	Honeybee	0.75	7.6	171	**0.102**	0.03
	Tomatoes		1.0	7.6	171	**0.135**	0.03

HQ/ETRs in **bold** are above the relevant trigger and require further refinement

The **$ETR_{chronic\ adult\ oral}$** values for chlorothalonil and A14111B are above the trigger of 0.03 for downward sprays, according to EFSA 2014, indicating that a tier 1 assessment is necessary.

Larval assessment

The larval screening step assessment is calculated by multiplying the application rate in kg a.s./ha by a shortcut value as defined in the guidance. The resulting exposure value is then divided by the larval NOEL value (µg a.s./larvae/development period) taken from an 8 day larval study. The subsequent **ETR_{larvae}** is considered to demonstrate acceptable risk where it is less than the applicable trigger value.

ETR_{larvae} = application rate (kg a.s./ha) x SV / NOEL (µg a.s./larvae/development period)

ETR_{larvae} values for exposure to chlorothalonil following use of A14111B according to the proposed uses are given in the table below.

Table 10.3.1-8: Screening step – Risk assessment of larval exposure to chlorothalonil and A14111B

Test	Crop	Species	App.	Shortcut	NOEL or	ETR_{larvae}	Trigger

substance	Group		rate (kg a.s./ha)	Value (downward spray)	LD_{10} (µg larva/dev period)		
Chlorothalonil	Cereals	Honeybee	0.75	4.4	10*	**1.32**	0.2
	Tomatoes		1.0	4.4	10*	**1.76**	0.2
A14111B	Cereals		2.286	4.4	31.3	**1.29**	0.2
	Tomatoes		3.048	4.4	31.3	**0.43**	0.2

HQ/ETRs in **bold** are above the relevant trigger and require further refinement

* LD_{10}

The screening level **ETR_{larvae}** values for chlorothalonil and A14111B are above the trigger of 0.2 for downward sprays, according to EFSA 2013, indicating that a tier 1 assessment is necessary.

Tier 1 risk assessment

Acute adult contact and oral assessment – Bumble bees

Table 10.3.1-9: Tier 1– Risk assessment of acute contact exposure to bumble bee

scenario	BBCH	Bumble bee	
		HQ	trigger
Cereals (0.75 kg/ha, BBCH 30-69)			
treated crop	30 - 39	<0.0	7
treated crop	≥ 40	<0.0	7
weeds	30 - 39	<3.8	7
weeds	≥ 40	<2.3	7
field margin	30 - 39	<0.2	7
field margin	≥ 40	<0.2	7
treated crop	30 - 39	<0.0	2.3
treated crop	≥ 40	<0.0	2.3
weeds	30 - 39	<0.4	2.3
weeds	≥ 40	<0.2	2.3
field margin	30 - 39	<0.7	2.3
field margin	≥ 40	<0.7	2.3
Tomato (1kg/ha, BBCH 51-89)			
treated crop	≥ 50	0.0	7
weeds	≥ 50	3.0	7
field margin	≥ 50	0.3	7
treated crop	≥ 50	0.0	2.3
weeds	≥ 50	0.3	2.3
field margin	≥ 50	1.0	2.3

The **$HQ_{contact}$** values for chlorothlaonil are less than the triggers of 7 and 2.3 for bumble bees, according to EFSA 2014, indicating that acute contact risk to bumble bees is acceptable following use of A14111B according to the proposed use pattern.

Table 10.3.1-10: Tier 1– Risk assessment of acute oral exposure to bumble bee

category	scenario	BBCH	Bumble bee

			ETR	trigger
Cereals (0.75 kg/ha, BBCH 30-69)				
acute	treated crop	30 - 39	0.02	0.036
acute	treated crop	40 - 69	0.02	0.036
acute	weeds	30 - 39	0.03	0.036
acute	weeds	40 - 69	0.02	0.036
acute	field margin	30 - 39	0.00	0.036
acute	field margin	40 - 69	0.00	0.036
acute	adjacent crop	30 - 39	0.00	0.036
acute	adjacent crop	40 - 69	0.00	0.036
acute	next crop	30 - 39	0.01	0.036
acute	next crop	40 - 69	0.01	0.036
Tomato (1kg/ha, BBCH 51-89)				
acute	treated crop	50 - 69	0.02	0.036
acute	treated crop	≥ 70	0.00	0.036
acute	weeds	50 - 69	0.02	0.036
acute	weeds	≥ 70	0.02	0.036
acute	field margin	50 - 69	0.00	0.036
acute	field margin	≥ 70	0.00	0.036
acute	adjacent crop	50 - 69	0.00	0.036
acute	adjacent crop	≥ 70	0.00	0.036
acute	next crop	50 - 69	0.01	0.036
acute	next crop	≥ 70	0.01	0.036

The **$ETR_{adult\ oral}$** values for chlorothalonil are less than the trigger of 0.036 for downward sprays, according to EFSA 2014, indicating that acute oral risk to bumble bees is acceptable following use of A14411B according to the proposed use pattern.

Chronic adult oral assessment

The chronic adult oral Tier 1 assessment is calculated by multiplying the application rate in kg a.s./ha by a shortcut value and the Exposure Factor (Ef) and the TWA, as defined in the guidance. The resulting exposure value is then divided by the chronic (10 day) adult oral LDD_{50} value (µg a.s./bee/day). The subsequent **$ETR_{chronic\ adult\ oral}$** is considered to demonstrate acceptable risk where it is less than the applicable trigger value.

$ETR_{chronic\ adult\ oral}$ = application rate (kg a.s./ha) x SV x Ef x TWA / $LDD_{50\ oral}$ (µg a.s./bee/day)

$ETR_{chronic\ adult\ oral}$ values for chronic exposure to chlorothalonil following use of A14111B according to the proposed uses are given in the table below.

Table 10.3.1-9: Tier 1– Risk assessment of chronic oral exposure to honeybee

Test substance	Crop Group	Scenario	App. rate (kg a.s./ha)	Shortcut Value (downward spray)	LDD_{50} oral (µg /bee/day)	Ef	TWA	$ETR_{chronic\ adult\ oral}$	Trigger

Chlorothalonil	Cereals	Treated crop	0.75	0.92	53.9	1	0.72	0.01	0.03
		Weeds		2.9		0.5		0.01	
		Field margin		2.9		0.0092		0.00	
		Adjacent crop		5.8		0.0033		0.00	
		Next crop		0.54		1		0.01	
A14111B		Treated crop	2.286	0.92	171	1		0.01	
		Weeds		2.9		0.5		0.01	
		Field margin		2.9		0.0092		0.00	
		Adjacent crop		5.8		0.0033		0.00	
		Next crop		0.54		1		0.01	
Chlorothalonil	Tomato	Treated crop	1.0	0.92	53.9	1		0.01	
		Weeds		2.9		0.3		0.01	
		Field margin		2.9		0.0092		0.00	
		Adjacent crop		5.8		0.0033		0.00	
		Next crop		0.54		1		0.01	
A14111B		Treated crop	3.048	0.92	171	1		0.01	
		Weeds		2.9		0.3		0.01	
		Field margin		2.9		0.0092		0.00	
		Adjacent crop		5.8		0.0033		0.00	
		Next crop		0.54		1		0.01	

The **$ETR_{chronic\ adult\ oral}$** values for chlorothalonil are less than the trigger of 0.03 for downward sprays, according to EFSA 2013, indicating that chronic risk to honeybees is acceptable following use of A14411B according to the proposed use pattern.

Larval assessment

The larval Tier 1 assessment is calculated by multiplying the application rate in kg a.s./ha by a shortcut value and the Exposure Factor (Ef) and the TWA, as defined in the guidance. The resulting exposure value is then divided by the larval NOEL value (µg a.s./larvae/development period) taken from an 8 day larval study. The subsequent **ETR_{larvae}** is considered to demonstrate acceptable risk where it is less than the applicable trigger value.

ETR_{larvae} = application rate (kg a.s./ha) x SV / x Ef x TWA NOED (µg a.s./larva/development period)

ETR_{larvae} values for exposure to chlorothalonil following use of A14111B according to the proposed uses are given in the table below.

Table 10.3.1-10: Tier 1– Risk assessment of larval exposure to honeybee

Test substance	Crop Group	Scenario	App. rate (kg a.s./ha)	Shortcut Value (downward spray)	NOED or LD_{10} oral (µg./larva/ dev period)	Ef	TWA	$ETR_{chronic\ adult\ oral}$	Trigger
Chlorothalonil	Cereals	Treated crop	0.75	0.15	10.0*	1	0.85	0.01	
		Weeds		2.2		0.5		0.07	
		Field margin		2.2		0.0092		0.00	
		Adjacent crop		4.4		0.0033		0.00	
		Next crop		0.4		1		0.03	
A14111B		Treated crop	2.286	0.15	31.3	1		0.01	
		Weeds		2.2		0.5		0.07	
		Field margin		2.2		0.0092		0.000	
		Adjacent crop		4.4		0.0033		0.000	
		Next crop		0.4		1		0.02	
Chlorothalonil		Treated crop		0.15		1		0.01	
		Weeds		2.2		0.3		0.06	

	Tomato	Field margin	1.0	2.2	10.0*	0.0092		0.00	0.2
		Adjacent crop		4.4		0.0033		0.00	
		Next crop		0.4		1		0.03	
A14111B		Treated crop	3.048	0.15	31.3	1		0.01	
		Weeds		2.2		0.3		0.05	
		Field margin		2.2		0.0092		0.00	
		Adjacent crop		4.4		0.0033		0.00	
		Next crop		0.4		1		0.03	

* LD_{10}

The **ETR_{larvae}** values are less than the trigger of 0.2 for downward sprays, according to EFSA 2014, indicating that the risk to honeybee larvae is acceptable following use of A14111B according to the proposed use pattern.

An additional study in honey bee larvae is available from the public literature. Zhu et. al. (2014), determined a lower LC_{50} (< 5.44 µg a.s./bee) than that which was found from the fully reliable study submitted with the dossier. Since this value is lower than the LD_{10} value used in the risk assessment above, the RMS must consider (1) the reliability of the endpoints and (2) the conservativeness of the risk assessment, overall. The dossier study was considered fully reliable, and the study from the public literature was also considered well performed, though several drawbacks were noted, including the fact that (1) standard Guidelines were not followed, (2) only a limit, nominal concentration was used, (3) there was no positive control, (4) mortality in the blank and solvent controls (pooled) was > 15% (17.2%). Further, the test was performed using chlorothalonil, whereas the dossier test was performed using the formulation. Zhu et. al. (2014) used acetone and methanol (1%) to dissolve the technical a.s. into the larval diet, and included two solvent controls and an untreated control, which did not show significantly different levels of larval mortality. Thus, it seems unlikely that the presence of solvent has resulted in the increased toxicity to larvae and it can be concluded that the formulation is therefore of lower toxicity. The exposure profiles are also quite different. In Zhu, et. al., it is stated that the larvae were placed on treated diet (i.e. Day 1), but also that old diet was removed and new diet provided on Days 1, 2, 3, 4, and 5. It is presumed that no replacement was necessary on day 1, but it is therefore not clear what the dosing actually was in this study. Since the test with the formulation was fully acceptable, the risk to honey bee larvae from A14111B is considered addressed by the formulation study.

Metabolite R182281 according to EFSA (2014)

R182281 (SDS-3701) is a plant metabolite and so needs consideration for potential risk to bees as there may be exposure through pollen and nectar.

R182281 is evaluated following the EFSA scheme for metabolites below, answers in **bold**

1. *Identify plant metabolites from plant metabolism studies in which the parent substance is applied in the same way as for the intended use. For the following crops this should include application to bare soil. Please note that if data on occurrence of metabolites in pollen and nectar are available, then the assessment should focus on these metabolites and it is not necessary to test other plant metabolites identified in the plant metabolism studies. Are there any identified metabolites formed in amounts of > 10% (TRR) or 0.01 mg/kg?*

R182881 is the major plant metabolite, found at levels > 0.01 mg/kg in plant metabolism studies – so

Yes: Go to 2.

No: No further assessment is required.

2. *Is it clear that the toxophore relevant for the toxicity to bees has been lost from the molecule (see Note 1)?*

Note 1: Identification of toxophore

Substances that have a specific mode of action, such as pesticides, contain a structural feature or moiety that gives the toxic property. This structural feature is referred as the toxophore, or toxophoric moiety. The substance causes toxicity through the interaction of its toxophore with a biomolecular site (e.g. receptor). Substances that are structurally similar could contain the same toxophore (or may yield a common toxophore upon metabolism) and may therefore have a common toxic effect.

For the assessment of the metabolite it may be possible for the applicant to provide a reasoned case as to whether the molecule contains a toxophore or it has been lost following transformation.

Toxophores for each of the major classes of PPPs have been identified by looking for substructural similarities within a pesticidal class by Sinclair (2009), which can be used to support argumentation. A number of ways have been identified to define domain of applicability, which may be used to decide whether or not toxophores are present (Dimitrov et al., 2005; Jaworska, 2005; Netzeva, 2005; Nikolova and Jaworska, 2005). If cannot be clearly shown that the toxophore is not present in the molecule, it should be assumed that the toxophore remains and that the molecule has a specific mode of action.

For chlorothalonil, the toxaphore is not obvious, but consideration can be given as to whether it has been lost.

R182281 shows practically no pesticidal activity relative to the parent chlorothalonil. A screening assay (K-CA 8.7/08) demonstrated that when R182281 is screened at concentrations related to the field equivalent rate of chlorothalonil in a glasshouse environment, it does not demonstrate antifungal activity against *Botrytis cinerea, Phytophthora infestans* or *Stagonospora nodorum.* Some activity,

inferior to chlorothalonil was detected against *Puccinia recondita*. These screening assays are not particularly discriminative, designed to pick up activity rather than show a lack of activity. The basis for the biological activity of chlorothalonil is glutathione reactivity. R182281 shows practically no activity ($<10^6$ x) relative to chlorothalonil (K-CA 8.7/11 Pierce A (2001). *GSH Reactivity of Chlorothalonil and Metabolites R182281, R419492 and R471811*).

RMS Conclusion: R182281 has apparently lost the significant biological activity and thus the notifier concludes that it has lost any toxophore of the parent, however the elements of the parent which provide the pesticidal mechanism of action are not necessarily the same as those which could cause toxicity to other non target organisms. Thus, the RMS does not consider this point to have been proven merely by showing that the biological and pesticidal actions of chlorothalonil have been lost, since the mechanism of fungicidal action is different from the mechanism of toxicological action. However, considering the aquatic data, the most sensitive species, *Mysidopsis bahia,* has been tested with the metabolite R182281 (SDS-3701) and it was found to be significantly less toxic than the parent (19 mg/L vs 0.0004 mg/L). Considering this, and the fact that the parent does not show significant toxicity to bees, the RMS agrees with the notifier that it is most likely that any potential toxophore for bees from chlorothalonil has been lost in the metabolite R182281.

Yes: No further assessment is required.
No or unclear: Go to 3.

Thus following the guidance, R182281 (SDS-3701) requires no further evaluation, as it has lost the toxaphore for fungicidal/biological activity of chlorothalonil and is significantly less toxic to aquatic invertebrates than the parent, chlorothalonil.
However, the guidance contains a method for metabolites which require further assessment of assuming 10X the toxicity of the parent. If any further proof of the low risk to bees from R182281 were needed, as plant residue data show maximum residues of R182281 as being <10x those of the parent (based on max residues not TRR, which is the correct way of assessing relative risk), then the metabolite presents an acceptable risk.
The low exposure of bees to R182281, compared to parent is confirmed by a recent study (K-CP 10.3.1/01), shown below, which looked at residues of both chlorothalonil and R182281 in pollen and nectar in cucumber, a representative bee attractive crop. In pollen, residues of R182281 were much less than 10% of the parent, ranging from <0.01 – 0.41 mg/kg in pollen compared to the parent 0.03 – 31 mg/kg. For nectar residues of parent were significantly lower than pollen, ranging from 0.01- 3.2 mg/kg and R182281 residues were also lower in nectar than pollen but more similar to the pollen value at <0.01 – 0.31 mg/kg.
RMS conclusion: The residue study confirms that the residues of R182281 in pollen and nectar are significantly (10x) lower than the parent, up to and including day 9 after the second application.The data from the residues section suggests that the highest levels of this metabolite in plant matter are typically found on day 1 or 0 after application. The residues study was performed in cucumber and is thus not fully applicable to the requested uses, however, as it is used as supporting information that

the metabolite will not be significantly toxic to bees, the RMS considers it sufficient to address this question. The risk to bees from the metabolite R18221 (SDS-3701) is considered addressed.

B.9.6.2 Risk assessment for arthropods other than bees

The risk assessment for non-target arthropods has been conducted in line with the Terrestrial Guidance Document (SANCO/10329/2002).

Toxicity

In table B.9.6.2-01 the endpoints for non-target arthropods of the formulation under assessment, A14111B, are presented.

Table B.9.6.2-01a: Table of Tier 1 laboratory test endpoints to assess risk from use of A14111B

Test Substance	Species	End point	Toxicity
A14111B, Tier 1 laboratory test	*Typhlodromus pyri*	Mortality, LR50 Reproduction, ER50	>2.095 kg chlorothalonil/ha <0.524 kg chlorothalonil/ha
A14111B, Tier 1 laboratory test	*Aphidius rhopalosiphi*	Mortality, LR50 Reproduction, ER50	>0.262 kg chlorothalonil/ha >1.048 kg chlorothalonil/ha

Table B.9.6.2-01b: Table of Tier 2 extended laboratory test endpoints to assess risk from use of A14111B

Species	Life stage	Test substance, substrate	Time scale	Dose (kg as/ha)[1,2]	End point	% effect[3]	LR_{50}
Typhlodromus pyri	protonymphs	A14111B, bean leaf discs (2-D)	14 d	0.017 0.084 0.419 2.095 0.017 0.084 0.419 2.095	Mortality Reproduction	0 0 0 12 -1 9 36 58	LR50 > 2.095 kg chlorothalonil/ha ER50 = 1.187 kg chlorothalonil/ha

Species	Life stage	Test substance, substrate	Time scale	Dose (kg as/ha)[1,2]	End point	% effect[3]	LR_{50}
Aphidius rhopalosiphi	adult	A14111B, barley seedlings (3-D)	48 h	0.524 1.048 2.095 0.524 1.048 2.095	Mortality Reproduction	0 0 0 17 27 9	LR50 > 2.095 kg chlorothalonil/ha ER50 > 2.095 kg chlorothalonil/ha
Chrysoperla carnea	larvae	A14111B, bean leaves (2-D)	48 h	0.017 0.084 0.419 1.048 2.095 0.017 0.084 0.419 1.048 2.095	Mortality Reproduction	16 10 19 15 7 0 0 0	LR50 > 2.095 kg chlorothalonil/ha ER50 > 2.095 kg chlorothalonil/ha

First tier risk assessment

The proposed field uses of A14111B lead to the following estimated exposure levels for use in a first tier risk assessment.

Table B.9.6.2-02 First tier estimated exposure levels from the proposed field uses of A14111B

Crop	Rate of use (mL prod/ha)	MAF	In-field exposure (mL prod/ha)	Drift rate	Veg. Distribu-tion factor	Correc-tion factor	Off-field exposure (mL prod/ha)
Cereals	1875	1.7	3188	0.0238	10	10	75.9
Tomatoes	2500	-	2500	0.0802			200.5

MAF: multiple application factor

In-field risk

The in-field risk to non-target arthropods was assessed by calculating Hazard Quotients (HQs) for the two sensitive indicator species, *T. pyri* and *A. rhopalosiphi*, using the following equation:

$$\text{In - field HQ} = \frac{\text{PER}_{\text{in-field}}\ (\text{mL/ha})}{\text{LR}_{50}\ (\text{mL/ha})}$$

The resulting HQ values are presented, to 2 significant figures, in the table below. When using Tier I data the risk is considered to be acceptable if the HQ is less than 2.

Table B.9.6.2-03: In-field HQs for non-target arthropods

Crop	Species	LR_{50} (mL prod/ha)	In-field foliar exposure	
			PER (mL prod/ha)	HQ
Cereals	*A. rhopalosiphi*	>625	3188	**<5.1**
	T. pyri	>5000		<0.64
Tomatoes	*A. rhopalosiphi*	>625	2500	**<4.0**
	T. pyri	>5000		<0.50

The in-field foliar HQ values for *T. pyri* are <2 for both crops, indicating an acceptable risk. However, the in-field foliar HQ values for *A. rhopalosiphi* are possibly above the trigger of 2 for both crops, indicating the need for further refinement. A higher tier risk assessment has therefore been conducted and is presented below.

Although not required by ESCORT 2 guidelines, fecundity was also assessed in the Tier I tests with the standard test species. In the *T. pyri* study effects of >50% were observed at 1250, 2500 and 5000 mL/ha. In the study conducted with *A. rhopalosiphi* no effects of >50% were observed at rates up to and including 2500 mL/ha. Unacceptable effects of reproduction cannot be excluded and therefore a higher tier risk assessment has been conducted and is presented below.

Refined in-field risk assessment

Extended laboratory tests (Tier II tests) have been conducted with *T. pyri, A. rhopalosiphi* and *C. carnea*. Results from these studies are summarised in Table B.9.6.2-01b.

The higher tier risk assessment is conducted according to ESCORT 2 guidance and uses a trigger value of 50% effect on lethal or sublethal endpoints in extended laboratory studies. If the LR_{50}, or sublethal 50% effect level value is greater than the PER value then no unacceptable effects would be predicted in-field following the use of A14111B in accordance with the uses supported in this submission.

The in-field assessment is presented in the table below.

Table B.9.6.2-04: In-field risk assessment for non-target arthropods

Crop	Test species	Endpoints (mL A4111B/ha)		PER (mLprod/ha)	Acceptable risk
Cereals	*T. pyri*	LR_{50}	>5000	3188	No
		$ER_{50\ (reproduction)}$	2833		
	A. rhopalosiphi	LR_{50}	>5000	3188	Yes
		$ER_{50\ (reproduction)}$	>5000		
	C. carnea	LR_{50}	>5000	3188	Yes
		$ER_{50\ (reproduction)}$	>5000		
Tomatoes	*T. pyri*	LR_{50}	>5000	2500	No
		$ER50_{(reproduction)}$	2833		
	A. rhopalosiphi	LR_{50}	>5000	2500	Yes
		$ER50_{(reproduction)}$	>5000		
	C. carnea	LR_{50}	>5000	2500	Yes
		$ER50_{(reproduction)}$	>5000		

The assessment presented in Table B.9.6.2-04 indicates that the LR_{50} values for all three species are in excess of the majority of the PER values indicating an acceptable risk. There were no unacceptable (>50%) effects on fecundity with *A. rhopalosiphi* or *C. carnea* at rates up to 5000 mL/ha, i.e. greater than the foliar PER values. For the use in tomatoes also the ER50 for *T.pyri* is in excess of the PER value. However, for *T. pyri*, the PER for the use in cereals is somewhat higher than the ER50 value. Hence, a reproductive risk to *T. pyri* cannot be excluded. Aged-residue studies are not available. However, taking into account that the PER is just above the ER50, that chlorothalonil is not a persistent compound and that the maximum frequency is not more than 2, an accceptable in-field risk with respect to the use in cereals is expected, keeping in mind that the acceptable recovery period in-field may be one year.

Off-field risk assessment

The off-field risk to non-target arthropods was assessed by calculating Hazard Quotients (HQs) for the two sensitive indicator species, *T. pyri* and *A. rhopalosiphi*, using the off-field exposure values as presented in table Table B.9.6.2-05.

For the standard species *T. pyri* and *A. rhopalosiphi* LR50 values of >5000 respectively >625 mL prod/ha are available from standard laboratory studies. With these values off-field HQ values can be calculated.

The resulting HQ values are presented, to 2 significant figures, in the table below. When using Tier I data the risk is considered to be acceptable if the HQ is less than 2.

Table B.9.6.2-05: Off-field HQs for standard species

Crop	Species	LR_{50}	Off-field foliar exposure

		(mLprod/ha)	PER (mLprod/ha)	HQ
Cereals	*A. rhopalosiphi*	>625	75.9	<0.12
	T. pyri	>5000		<0.02
Tomatoes	*A. rhopalosiphi*	>625	200.5	<0.32
	T. pyri	>5000		<0.04

From the table it appears that the off-field risk *A. rhopalosiph*i for *T. pyri* is acceptable for both applications (HQ < 2).

Although not required by ESCORT 2 guidelines, fecundity was also assessed in the Tier I tests with the standard test species. In the *T. pyri* study effects of >50% were observed at 1250, 2500 and 5000 mL/ha. In the study conducted with *A. rhopalosiphi* no effects of >50% were observed at rates up to and including 2500 mL/ha. Unacceptable effects of reproduction cannot be excluded and therefore a higher tier risk assessment has been conducted and is presented below.

Refined off-field risk assessment

Extended laboratory tests (Tier II tests) have been conducted with *T. pyri, A. rhopalosiphi* and *C. carnea*. Results from these studies are summarised in Table B.9.6.2-01b.

The higher tier risk assessment is conducted according to ESCORT 2 guidance and uses a trigger value of 50% effect on lethal or sublethal endpoints in extended laboratory studies. If the LR_{50}, or sublethal 50% effect level value is greater than the PER value then no unacceptable effects would be predicted off-field following the use of A14111B in accordance with the uses supported in this submission.

The off-field assessment based on extended lab studies is presented in the table below.

Table B.9.6.2-06 Off-field HQs for standard species

Crop	Rate of use	MAF	Drift rate	Veg. distribution factor	Correction factor	Off-field exposure (mL prod/ha)	L(E)R 50 (ml prod/ ha)*	HQ (trigger is 1)
***Typhlodromus pyri*: 2D test-system**								
Cereals	2 x 1875 mL prod/ha	1.7	0.0238	10	5	37.9	2833	0.013
Tomatoes	1 x 2500 mL prod/ha	n.a.	0.0802			100.3	2833	0.035

Crop	Rate of use	MAF	Drift rate	Veg. distribution factor	Correction factor	Off-field exposure (mL prod/ha)	L(E)R50 (ml prod/ha)*	HQ (trigger is 1)
***Aphidius rhopalosiphi*: 3D test-system**								
Cereals	2 x 1875 mL prod/ha	1.7	0.0238	n.a.	5	379	>5000	<0.076
Tomatoes	1 x 2500 mL prod/ha	n.a.	0.0802			1002.5	>5000	<0.20
***Chrysoperla carnea:* 2D test-system**								
Cereals	2 x 1875 mL prod/ha	1.7	0.0238	10	5	37.9	>5000	<0.008
Tomatoes	1 x 2500 mL prod/ha	n.a.	0.0802			100.3	>5000	<0.02

n.a. = not applicable
* the lowest value of the LR50 and ER50 values are presented

The assessment presented in Table B.9.6.2-06 indicates that the LR_{50} and ER_{50} values for all three species are in excess of the of the PER values indicating an acceptable off-field risk.

B.9.7 Effects on non-target soil meso- and macrofauna

B.9.7.1 Earthworms

B.9.7.1.1 Acute toxicity to earthworms

Report:	IIIA, 10.3.5.1/01 (numbering of Volume 2 original DAR). Wütrich, V 1990. Acute toxicity (LC_{50}) study of Daconil 2787 Extra to earthworms. Generated by: RCC Umweltchemie AG Submitted by: Zeneca Report No.: 282971 Date: November 29, 1990 GLP, Unpublished
Previous evaluation	In DAR (2000) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Species	Duration	MC [% w/w]	o.m. [%]	pH	T [°C]	Criterion	Value	Unit	Ri
Daconil 2787 Extra	Eisenia foetida	14 days	32-46	10	7.4	21-23	LC50	>1000	mg/kg	1

Description

Daconil 2787 Extra is a suspension concentrate containing 40.4% chlorothalonil. Five test concentrations, 62.5- 1000 mg/kg dw, plus control. Distilled water as vehicle. Worms 200-228 mg at start. Test according to OECD 207 Guidelines.

Results

No mortalities up to 500 mg/kg; 45% at 1000 mg/kg. LC50 >1000 mg/kg bw. Treatment-related influence on body weights at 500 mg/kg and higher: 20-34%. NOEC 250 mg/kg dw.

Remarks

This study was submitted for both Annex II and III. With Spearman-Karber no LC50 can be calculated. The result is used for risk evaluation.

B.9.7.1.2 Chronic toxicity to earthworms

Report: K-CP 10.4.1.1/01 Staebler D. (2004). Azoxystrobin / Chlorothalonil (ZA5504 / RO44686) 80/400 g/L SC formulation (A14111B): Sublethal toxicity of a 480 g/L SC formulated mixture to the earthworm *Eisenia fetida* in artificial soil. Report Number 20031441/01-NREf. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. (Syngenta file No. ICI5504/2303)	
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. The NOEC was determined to be 80 mg A14111B/kg dry weight soil (corresponding to 27.5 mg chlorothalonil/kg dry weight soil). The EC_{50} was > 80 mg A14111B/kg dry weight soil (corresponding to > 27.5 mg chlorothalonil/kg dry weight soil). No meaningful values for reproduction EC_{10} and EC_{20} could be derived.

Guidelines

ISO 11268-2 (1998) and draft OECD 222 (January 2000)

GLP: Yes.

Executive Summary

In a 56-day study determining the sublethal toxicity of A14111B to *E. fetida* earthworms, the NOEC for weight change and reproduction was 80 mg A14111B/kg soil dry weight (the highest tested concentration).

Materials

Test Material:	Azoxystrobin/chlorothalonil SC (80/400) (formulation code A14111B)
Description:	Opaque cream-coloured liquid, nominally containing 80 g/L

	azoxystrobin and 400 g/L chlorothalonil
Lot/Batch #:	J7518/024
Purity:	80 g/L (corresponding to 6.6 % w/w) azoxystrobin and 419 g/L (corresponding to 34.6 % w/w) chlorothalonil
Stability of test compound:	Assumed stable pending re-analysis in September 2005
Vehicle and control:	Deionised water
Toxic reference:	Derosal flüssig, a.s. Carbendazim at 4 mg a.s./kg soil d.w.
Test organisms	
Species:	*Eisenia fetida* (Oligochaeta: Lumbricidae)
Source:	GAB Biotechnologie internal laboratory source
Test substrate:	Artificial soil (OECD 222, 10% O.M. and water holding capacity 50.2 mL/100g)
Environmental test conditions	
Temperature:	20± 2°C
pH	6.1 – 6.5
Photoperiod:	16:8 light:dark artificial light (approx.. 650 lux)

Study Design and Methods

Experimental dates: 8th January to 4th March 2004.

An aqueous preparation of A14111B in deionised water was applied to artificial soil to give test concentrations of 5, 10, 20, 40 and 80 mg formulation/kg dry weight of soil (corresponding to 1.7, 3.3, 6.6, 13 and 26 mg chlorothalonil/kg soil dry weight), plus control. Four replicates per treatment were prepared and 10 clitellate adult earthworms (*Eisenia fetida*, between 2 months and 1 year old with a clitellum and a body weight between 300 – 400 mg each at start of the study) added to each replicate. After 4 weeks the adult worms were removed, weighed and checked for clinical symptoms. The soil, including the offspring, was returned to the test vessels for another 4 weeks exposure. Eight weeks after test start, the test was terminated and the exact number of juvenile worms was determined per test vessel and treatment group. The reported test endpoints were NOEC and LOEC for weight change and reproduction. The RMS added the EC_{50} estimation and a statement on the EC_{10} and EC_{20} for reproduction.

Results and Discussion

The results of the long-term effects of A14111B on earthworms are summarised in Table 10.4.1.1-1.

Table 10.4.1.1-1: A14111B - sub-lethal toxicity to earthworms

Treatment	Treatment concentration (mg/kg soil dry weight)					
	Control	5	10	20	40	80
Adult Mortality (%)	0	0	0	0	0	0[a]
Adult Weight change (%)	+ 7.3	+ 10.5	+ 6.3	+ 9.2	+ 16.5	+ 6.5
Mean number of juveniles	68	74	79	82	59	81
	Endpoints					
NOEC	80 mg/kg soil dry weight					
LOEC	≥ 80 mg/kg soil dry weight					

[a] 1 adult earthworm escaped during test period

No effects on behaviour (including feeding activity) and no pathological symptoms of the worms were observed during the test. The test item caused no statistically significant change in worm growth (change in fresh weight after 4 weeks relative to initial fresh weight) relative to the control treatment at any concentration tested. No statistically significant effects on the number of juveniles compared to the control group were recorded at any concentration. The EC_{50} for reproduction was > 80 mg A14111B/kg dry weight soil (corresponding to > 27.5 mg chlorothalonil/kg dry weight soil). As no clear dose -response was evident in the mean number of juveniles, no meaningful EC_{10} and EC_{20} values for reproduction could be derived.

The reproduction capacity in the reference item group was statistically significantly reduced compared to the control (mean value of 2 juvenile worms per replicate corresponding to 97% reduction). This is in line with the OECD 222 guideline.

Validity criteria of the OECD 222 (2004) were met:

- Adult mortality in the controls over the initial 4 weeks was ≤ 10 % (i.e. 0 %)
- Each replicate (containing 10 adults) of the control had produced ≥ 30 juveniles by the end of the test (i.e. 46-81)
- The coefficient of variation of reproduction in the controls was ≤ 30 % (i.e. 23.5%)

Conclusion

The no-observed-effect-concentration (NOEC) was determined to be 80 mg A14111B/kg dry weight soil (corresponding to 27.5 mg chlorothalonil/kg dry weight soil). The EC_{50} was > 80 mg A14111B/kg

dry weight soil (corresponding to > 27.5 mg chlorothalonil/kg dry weight soil). No meaningful values for reproduction EC_{10} and EC_{20} could be derived.

7. Potter et al. (1990) Toxicity of pesticides to earthworms (Oligochaeta: Lumbricidae) and effect on thatch degradation in Kentucky bluegrass turf. Journal of Economic Entomology Vol 83, no. 6, 2362-69.

In this article a field study is described and it is found that at a dosage of 12.66 kg a.i./ha (as Daconil 2787 Extra) the number of earthworms (mainly *Aporrectodea spp*.) in the treated field is max. 25% lower than in the control field, but this reduction is statistically not significant. The maximum effect is found after one week, and after one month the effect is 16%. These results are comparable to other herbicides tested, and are insignificant compared to the effects of some insecticides.

B.9.7.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Report: KCP 10.4.2.1/01 Fauser-Misslin, A. (2015) Azoxystrobin/Chlorothalonil (A14111B) – Effects on reproduction of *Folsomia candida* (Collembola: Isotomidae), Report Number 20140139. Innovative Environmental Services (IES), Benkenstrasse 260, 4108 Witterswil, Switzerland (Syngenta File No Syngenta file No. A14111B_11214).	
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. The NOEC for mortality was determined to be 1000 mg A14111B/kg soil dry weight. The EC_{10}, EC_{20} and EC_{50} for number of juvenile collembolans were calculated to be 35, 81 and 291 mg A14111B/kg soil d.w, respectively (corresponding respectively to 12, 27 and 97 mg chlorothalonil/kg soil d.w.) and the NOEC for reproduction was 53 mg A14111B/kg soil d.w (corresponding to 18 mg chlorothalonil/kg soil d.w.).

Guidelines

OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

GLP: Yes.

Executive Summary

The toxicity of A14111B to the parental mortality and reproduction of collembola species *Folsomia candida* were determined. The NOEC for mortality was determined to be 1000 mg A14111B/kg soil dry weight. The EC_{50} for number of juvenile collembolans was estimated to be 291.26 mg A14111B/kg soil d.w and the NOEC was 53 mg A14111B/kg soil d.w.

Materials

Test Material Azoxystrobin/Chlorothalonil SC

A14111B

Lot/Batch #:	GRA4K222B
Actual content of active ingredients:	Azoxystrobin: 6.74 % w/w (82.4 g/L) Chlorothalonil: 33.3 % w/w (407 g/L)
Description:	Greyish liquid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of December 2017
Density:	1222 kg/m^3
Treatments	
Test rates:	16, 29, 53, 95, 172, 309, 556 and 1000 mg A14111B/kg soil d.w.
Control:	Deionised water
Toxic standard:	Boric acid (100.3 % purity) 200 mg boric acid/kg soil d.w.
Application method:	Stock solution was mixed with 160 g soil using a hand mixer
Test organisms	
Species:	*Folsomia candida*
Age:	Synchronised 9 to 12 day old juveniles
Source:	Culture maintained at Test Facility
Feeding:	*Ad libitum* supply of granulated baker's yeast throughout the study
Test design	
Arenas:	Glass containers (8.5 cm height x 4 cm diameter) covered with lids allowing gaseous exchange, filled with 30 g (wet weight) of artificial soil, soil depth was 2-4 cm.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74 % fine quartz sand and < 1.0 % calcium carbonate
Replication:	4 replicates per test item and reference item treatment, 8 replicates per control treatment
No./arena :	10 juveniles
Duration of test:	28 days after treatment application
Environmental test conditions	
Temperature:	18.1 – 21.5 °C
pH of soil:	6.1 – 7.1
Water content of soil:	Test intiation: 47.5 to 52.8 % WHC_{max} Test termination: 43.1 to 50.2 % WHC_{max}
Photoperiod:	16: 8 L:D 404 – 439 Lux

Study Design and Methods

Experimental dates: 29th January to 19th March 2015

The test concentrations were individually prepared by dispersing an exact volume of the test item in waterEach treatment solution was thoroughly mixed with 160 g artificial soil using a hand stirrer, achieving a final nominal water content of 40-60 % of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates were used per test and reference item concentration and eight replicates for the control. The test organisms were fed with granulated dry yeast *ad libitum* throughout the test duration. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The LC and EC data were determined with Weibull analysis using linear maximum likelihood regression. Reproduction data were tested for normal distribution and homogeneity of variance, then with a Step-down Cochran-Armitage test and William's Multiple Sequential t-test to determine the NOEC values for mortality and reproduction respectively.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 10.4.2.1-1: Effects of residues of A14111B on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg A14111B/kg soil d.w.)								
	Control	**16**	**29**	**53**	**95**	**172**	**309**	**556**	**1000**
% Mortality of parental collembolans after 4 weeks	2.5	0.0	2.5	0.0	2.5	0.0	0.0	2.5	2.5
Mean number of juveniles after 4 weeks	1626	1513	1441	1537	1306[a]	1021[a]	750[a]	351[a]	369[a]
SD	167	109	63	107	187	273	85	121	99
CV %	10	7.2	4.3	7	14	27	11	35	27
NOEC (mortality)	1000 mg A14111B/kg soil d.w.								
NOEC (reproduction)	53 mg A14111B/kg soil d.w.								
LC_{50}	> 1000 mg A14111B/kg soil d.w.								
EC_{10}	35 mg A14111B/kg soil d.w.								
EC_{20}	81 mg A14111B/kg soil d.w.								
EC_{50}	291 mg A14111B/kg soil d.w.								

[a]: Statistically significantly lower when compared to the control (Williams Multiple Sequential t-test, α = 0.05, one-sided smaller)

CV: Coefficient of variance

The reproduction capacity in the reference item group was reduced by more than 50% as compared to the control (mean number of juveniles after 4 weeks was 607 which is a reduction of 63%) at 200 mg boric acid/kg soil d.w. This is in line with the EC50 value of 147 mg/kg soil from the ISO 11267 (2014) guideline.

Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 2.5 % (must be < 20%)
- The mean number of juvenile recorded in the control treatment was 1626 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 10 % (must not be > 30%)

Conclusions

The toxicity of A14111B to the parental mortality and reproduction of collembola species *Folsomia candida* were determined. The NOEC for mortality was determined to be 1000 mg A14111B/kg soil dry weight. The EC_{10}, EC_{20} and EC_{50} for number of juvenile collembolans were calculated to be 35, 81 and 291 mg A14111B/kg soil d.w, respectively (corresponding respectively to 12, 27 and 97 mg chlorothalonil/kg soil d.w.) and the NOEC for reproduction was 53 mg A14111B/kg soil d.w (corresponding to 18 mg chlorothalonil/kg soil d.w.).

Report: KCP 10.4.2.1/02 Fauser-Misslin, A. (2015a) Azoxystrobin/Chlorothalonil (A14111B) - Effects on Reproduction of *Hypoaspis aculeifer* (Gamasida: Laelapidae) in Artificial Soil, Report Number 20140140. Innovative Environmental Services (IES), Benkenstrasse 260, 4108 Witterswil, Switzerland. (Syngenta File No. A14111B_11215).	
evious evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable as indicative data only. The NOEC for both mortality and reproduction were determined to be 1000 mg /kg soil d.w. (corresponding to 333 mg chlorothalonil/kg soil d.w.). The 14-day $LC_{50,}$ EC_{10}, EC_{20} and EC_{50} were all considered to be > 1000 mg /kg soil d.w. (corresponding to 333 mg chlorothalonil/kg soil d.w.).The positive control did not give the expected response, indicating that the sensitivity of the batch of soil mites tested may not have been sufficient. As a consequence, the results of this test will not be taken to the LoEP, and can be used as indicative data only.

Guidelines

OECD Guideline 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil (2008)

GLP: Yes.

Executive Summary

The effects of A14111B on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test. The NOEC for both mortality and reproduction were determined to be 1000 mg /kg soil dry weight, and the 14-day LC_{50} and EC_{50} could not be determined but were both considered to be > 1000 mg /kg soil dry weight, the highest concentration tested.

Materials

Test Material	Azoxystrobin/Chlorothalonil SC A14111B
Lot/Batch #:	GRA4K222B
Actual content of active ingredients:	Azoxystrobin: 6.74 % w/w (82.4 g/L) Fludioxonil: 33.3 % w/w (407 g/L)
Description:	Greyish liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of December 2017
Density:	1222 kg/m^3
Treatments	
Test rates:	16, 29, 53, 95, 172, 309, 556, 1000 mg A14111B/kg soil d.w.
Control:	Deionised water
Toxic standard:	Dimethoate (99.5 % purity) 7 mg dimethoate/kg soil d.w.
Test organisms	
Species	*Hypoaspis aculeifer* (CANESTRINI)
Source:	Cultured in test facility (originally: Katz Biotech AG, 15837 Baruth, Germany)
Food:	Cheese mites, *Tyrophagus putrescentiae*, 2-3 times per week
Age at test start:	28 to 35 days old
Test design	
Vessels:	Glass containers (volume: 100 mL; diameter: 4 cm; height 7.5 cm) with a lid allowing gaseous exchange, filled with 25 g wet weight of artificial soil.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74 % industrial quartz sand and < 1% calcium carbonate.
Replication:	Control group: 8 Treated group: 4

No. of mites/arena : 10 females

Duration of test: 14 days after treatment application

Environmental test conditions

Temperature: 18.1 to 21.4 °C

pH: Test start: 6.1 – 6.4

Test end: 6.2 – 6.5

Water content of soil: Test start: 49.1 to 53.4 % of WHC_{max}

Test termination: 45.3 to 51.0 % of WHC_{max}

Photoperiod: 16 h light : 8 h dark, 408 – 437 lux

Study Design and Methods

Experimental dates: 26th January to 9th March 2015

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to eight concentrations of A14111B incorporated into the test soil. Each treatment solution was prepared by diluting the proper amount of test item in 50 mL purified water after which 30 mL was hand-mixed with 120 g artificial soilsuch that, when added to pre-moistened artificial soil, a final moisture content value of 40 – 60 % WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.
Mortality and reproduction data were analysed using Williams Multiple Sequential t-tests (α = 0.05, one-sided smaller). Weibull analysis usinf linear maximum likelihood regression was used to determine LC_x and EC_x values.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 10.4.2.1-2: Effects of residues of A14111B on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A14111B/kg soil d.w.)								
	Control	**16**	**29**	**53**	**95**	**172**	**309**	**556**	**1000**
	Mortality of adult mites after 14 days								
% mortality	13	5	13	15	18	25	20	7.5	18

	Number of juveniles after 14 days								
Mean no. progeny per replicate	58	54	147	115	112	115	117	102	80
standard deviation	17	17	26	15	30	39	26	46	25
CV %	30	31	18	13	27	34	22	45	31
NOEC (mortality)	**1000 mg A14111B/kg soil d.w.**								
LC_{50} (mortality)	**> 1000 mg A14111B/kg soil d.w.**								
NOEC (reproduction)	**1000 mg A14111B/kg soil d.w.**								
EC_{50} (reproduction)	**> 1000 mg A14111B/kg soil d.w.**								
EC_{20} (reproduction)	**> 1000 mg A14111B/kg soil d.w.**								
EC_{10} (reproduction)	**> 1000 mg A14111B/kg soil d.w.**								

n.d.: Not determined due to low toxicity

CV: Coefficient of variance

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 13 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 58)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 30 %)

Conclusions

The effects of A14111B on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test. The NOEC for both mortality and reproduction were determined to be 1000 mg /kg soil dry weight (corresponding to 333 mg chlorothalonil/kg soil d.w.), and the 14-day $LC_{50,}$ EC_{10}, EC_{20} and EC_{50} could not be determined but were all considered to be > 1000 mg /kg soil dry weight (corresponding to 333 mg chlorothalonil/kg soil d.w.), the highest concentration tested.

Remarks RMS

The reproduction capacity in the reference item group was reduced by less than 50% as compared to the control (mean number of juveniles on day 14 was 37 corresponding to a reduction of 36%; reduction of reproduction per replicate 17, 84, 31 and 12%). The OECD 226 indicates that the EC_{50} for dimethoate based on the number of juveniles should fall between 3 and 7 mg a.s./kg soil d.w.

(concentration dimethoate in the test was 7 mg/kg soil d.w.). Therefore, the results for the positive control indicate that the sensitivity of the batch of soil mites tested was not as expected. As a consequence, the results of this test will not be taken to the LoEP, and can be used as indicative data only.

Report: KCA 8.4.2.1/01 Vinall S, (2014), Chlorothalonil SC (A7867A) – A laboratory test to determine the effects of fresh residues on the predatory mite, *Hypoaspis aculeifer* (Acari: Laelapidae). Report Number SYN-14-1. Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, UK. (Syngenta file No. A7867A_11243).	
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. The NOEC was 1000 mg A7867A/kg soil dry weight (corresponding to 399 mg chlorothalonil/kg soil dry weight), and the 14-day EC_{10}, EC_{20} and EC_{50} were >1000 mg A7867A/kg soil d.w. (corresponding to > 399 mg chlorothalonil/kg soil dry weight).

Guidelines

OECD (2008). OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 226 (adopted 3 October 2008): Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil.

GLP: Yes.

Executive Summary

The potential effects of A7867A on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) were determined during a 14-day test.

The NOEC was determined to be 1000 mg A7867A/kg soil dry weight (corresponding to 399 mg chlorothalonil/kg soil dry weight), and the 14-day EC_{10}, EC_{20} and EC_{50} were considered to be >1000 mg A7867A/kg soil d.w. (corresponding to > 399 mg chlorothalonil/kg soil dry weight), the highest concentration tested.

Materials

Test Material	A7867A	
	Chlorothalonil SC (500)	
Lot/Batch #:	CHE2C00035	
Actual content of active ingredients:	Chlorothalonil:	39.9 % w/w corresponding to 498 g/L
Description:	Off-white suspension	
Stability of test	Stable under standard conditions	

compound:	
Reanalysis/Expiry date:	30 April 2015
Density:	1248 kg/m^3
Treatments	
Test rates:	95.3, 171.5, 308.6, 555.6 and 1000 mg A7867A/kg soil dry weight
Control:	Untreated substrate, i.e. purified water only
Toxic standard:	BAS 152 11 I Perfekthion (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L) applied at a rate of 32.07 mg product/kg soil d.w. (12 mg a.i./kg soil d.w.)
Application method:	Solutions of A7867A with purified water were dispersed in pre-moistened artificial soil prior to introduction of mites
Test organisms	
Species	*Hypoaspis aculeifer* (CANESTRINI)
Source:	Originally obtained from ECT Oekotoxikologie GmbH, Germany, and maintained at the Test Facility
Food:	Every 1 – 3 days with *Tyrophagus putrescentiae* (SCHRANK) and juveniles of *Folsomia candida* (WILLEM)
Age at test start:	Approximately 7 – 14 days from becoming adult
Test design	
Vessels:	60 mL glass jars (5.5 cm tall x 5.2 cm outer diameter, 4.4 cm inner diameter) with screw tops, and a net-covered 8-mm-diameter hole in the tops for ventilation
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolin clay, 74.85 % industrial quartz sand and 0.15% calcium carbonate. 20 g (dry weight equivalent) of artificial soil was added to each test vessel
Replication:	Control group: 8 (+ 2 replicates for determination of water content and pH-value; not loaded with predatory mites) Treated group: 4 (+ 2 replicates for determination of water content and pH-value; not loaded with predatory mites)
No. of mites/arena :	10 females
Duration of test:	14 days
Environmental test conditions	
Temperature:	19.6 - 20.6 °C
pH:	Test start: 6.25 – 6.35 Test end: 5.90 – 6.07
Water content of soil:	50% of maximum WHC.

Photoperiod: 16 h light : 8 h dark, 550 - 650 Lux

Study Design and Methods

Experimental dates: 11 November 2013 to 20 January 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of A7867A incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with purified water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of 50% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control) or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food Cheese mites (*Tyrophagus putrescentiae*) and juvenile springtails (*Folsomia candida*) were added to the soil surface of each test arena, at the beginning of the test and *ad libitum* (every 1 - 3 days) throughout the test. The test was carried out under controlled light-dark cycle. The water content was adjusted at 7 days after treatment by carefully adding purified water to bring the water content back up to 50% WHC. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. Any adult mites not found after extraction were recorded as dead. From these data the mortality of the adult females and the reproductive output were calculated.

The percentage mortality at each treatment rate was corrected for mortality in the control treatment using Abbott's formula (Abbott, 1925) and the data compared to that for the control using Fisher's Exact Test. Probit regression analysis to derive the EC_{50} values could not be performed on the data for numbers of progeny in the test item, as none of the treatments resulted in ≥50% reduction on juvenile numbers, compared to the control. One way ANOVA and Dunnett's t-test were used to compare the control with the independent test item groups for numbers of F1 progeny, and the LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) were determined from this.

The percentage reduction of reproductive output (R) for the treatment groups relative to the control was calculated using the formula:

$$R = [1-(R_t/R_c)] * 100$$

where R_t and R_c are the absolute values observed in the treatment and control groups respectively.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-1: Effects of residues of A7867A on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A7867A/kg soil d.w.)						
	Control	**95.3**	**171.5**	**308.6**	**555.6**	**1000**	**Toxic reference**
	Mortality of adult mites after 14 days						
% mortality[a]	0	3	0	3	3	0	100*
% corrected mortality[b] (Abbott)[b]	-	3	0	3	3	0	100
	Number of juveniles after 14 days						
Mean no. progency per replicate [c]	321	317	312	319	334	304	10*
standard deviation	20.6	19.9	13.5	19.3	42.8	15.5	9.0
coefficient of variation %	6.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
% reduction compared to control[d]	-	1	3	1	-4	6	97

[a] Mortality amongst mites originally introduced. Individual test-item treatments compared to the control using Fisher's Exact Test (α = 0.05). Treatments that differed significantly from the control are indicated with an asterisk (*).

[b] Calculated using Abbott's formula for corrected mortality (Abbott, 1925): M (%) = (1-t/c)*100%

[c] The results for each treatment were individually compared to the control by one-way ANOVA and Dunnett's t-test (α = 0.05). Values marked with an asterisk (*) differed significantly from the control.

[d] A positive value indicates a decrease and a negative value indicates an increase in reproduction, relative to the control.

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of control adult females: ≤ 20% (0 % observed)
- Mean number of juvenile per replicate: ≥ 50 (321 observed)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (6.4 % calculated)

Additionally, the efficiency of the method used to extract the mites in this test should be >95%. In a separate test carried out by the Test Facility, this was determined to be 100% and 98.6% for adult females and juveniles, respectively.

Conclusions

The NOEC was determined to be 1000 mg A7867A/kg soil dry weight (corresponding to 399 mg chlorothalonil/kg soil dry weight) and the 14-day EC_{10}, EC_{20} and EC_{50} wereconsidered to be >1000 mg A7867A/kg soil d.w. (corresponding to > 399 mg chlorothalonil/kg soil dry weight), the highest concentration tested.

B.9.8 Risk assessment for non-target soil meso- and macrofauna

B.9.8.1 Risk assessment earthworms

In Volume 1, section 2.9.4-1 an overview of the available endpoints for earthworms is given.

The endpoints for chlorothalonil have been divided by 2, because the log Pow is higher than 2 irrespective of whether the tests were conducted in artificial soil containing 5% peat. The log Pow of the metabolites is lower than 2, hence, no correction is necessary. The highest PECs, whether it was the initial or peak accumulation value has been used for the assessment.
Ten chlorothalonil metabolites are relevant in soil: R182281 (SDS-3701), R417888 (VIS-01), R418503, R419492, R471811, SYN507900 (SDS-66882), R611965 (SDS-46851), R611966 (SDS-47523), R611967 (SDS-47524) and R613636 (SDS-19221).

However, only for three metabolites acute toxicity data are available and for two metabolites chronic sdata are submitted. The notifier has submitted the following statement regarding metabolites of chlorothalonil:
*The occurrence of potentially ecotoxicologically relevant metabolites has been considered and are discussed in **M-CP Section 9.** Soil organisms could potentially be exposed to soil metabolites, as could aquatic organisms. In addition, the EFSA Aquatic Guidance states that the sediment/water metabolism and the aerobic mineralistation in surface studies should be considered to identify potentially ecologically relevant metabolites. A large amount of data are available to assess the risk from the metabolites, including ecotoxicological testing, fungicidal activity, as well as glutathione reactivity (the basis of the biological activity of chlorothalonil). Environmental metabolism generally involves the replacement of one or more of the Cl or CN groups. Although highly complex there are clear structural similarities between many of the metabolites of chlorothalonil. This was recognised in the EU Assessment Report and agreed that for risk assessment purposes R182281, R611965 and R417888 represented the major structural groupings. Accordingly, as is the case for toxicological purposes, it is considered that R417888 and R611965 cover the other sulphonic and carboxylic acid metabolites for ecotoxicology. The water sediment study identified metabolites not found in the soil metabolism, R613841, R613842 and R613801, these have also been tested for toxicity to aquatic organisms. All the relevant soil and water metabolites that have been tested are of much lower toxicity than the parent to aquatic organisms. Soil metabolites have been shown to be of similarly low toxicity or lower toxicity than the parent to soil organisms. None of the potential soil metabolites tested have shown any biological activity in fungicidal testing (R182281, R417888, R611965, R613636, R611968,, SYN507900, R419492, R471811, R418503, SYN548008, SYN548580 and SYN548581) or*

glutathione recativity (R182281, R417888, R611965, R613636, R611968, SYN548580, SYN548581, SYN548008, R419492, R471811), which is the biological basis for chlorothalonil's activity and so would not be expected to show any significant non-target toxicity.

The RMS is of the opinion that the argumentation of the notifier regarding the soil metabolites is not sufficiently convincing. Further argumentation or data on soil metabolites not tested yet, regarding the risk to earthworms, are considered necessary.

The notifier submitted chronic toxicity data on the metabolites R418503, R419492, R471811, SYN507900, R611965, R611966, R611967 and R61363.

The acute and chronic toxicity values of the active substance and the metabolites are compared with the PECsoil values from section B.8.

The acute and chronic TER values are presented in Table 9.8.1-01 and -02.

Table 9.8.1-01 TER calculations for earthworms – acute risk

Compound	Time scale (days)	LC50corr (mg a.s./metabolite / kg soil)	Max initial PEC_{soil} (mg as/kg soil)	TER	Trigger
cereals, 2x 750 g a.s./ha, interval 14 days					
Chlorothalonil	14	268.5	0.342	785	10
R182281	14	585	0.351	>1000	10
R417888	14	>1000	0.338	>1000	10
R611965	14	>1000	0.239	>1000	10
Tomatoes, 1x 1000 g a.s./ha					
Chlorothalonil	14	268.5	0.267	>1000	10
R182281	14	585	0.234	>1000	10
R417888	14	>1000	0.225	>1000	10
R611965	14	>1000	0.159	>1000	10

The table above shows that the acute risk to earthworms is acceptable for chlorothalonil and the metabolites R182281, R417888 and R611965.

Table 9.8.1-02 TER calculations for earthworms – chronic risk

Compound	Time scale (days)	NOECcorr (mg a.s./metabolite / kg soil)	Max initial PEC_{soil} (mg as/kg soil)	TER	Trigger
cereals, 2x 750 g a.s./ha, interval 14 days					
Chlorothalonil	56	2.5	0.342	7.3	5
A14111B	56	>13.8	0.342	>40.4	5
R182281	56	10	0.351	28.5	5
R417888	56	12.5	0.338	37.0	5
SYN548708 (R418503)	56	≥0.66	0.147	**≥4.49**	5
SYN548765 (R419492)	56	≥6.28	0.314	≥20	5
SYN548766 (R471811)	56	≥5.94	0.297	≥20	5

SYN507900	56	≥1.78	0.056	≥31.8	5
R611965	56	≥4.78	0.239	≥20	5
R611966	56	≥0.58	0.037	≥15.7	5
R611967	56	≥0.78	0.039	≥20	5
R613636	56	≥0.84	0.042	≥20	5
Tomatoes, 1x 1000 g a.s./ha					
Chlorothalonil	56	2.5	0.267	9.4	5
A14111B	56	>13.8	0.267	>51.7	5
R182281	56	10	0.234	42.7	5
R417888	56	12.5	0.225	55.6	5
SYN548708 (R418503)	56	≥0.66	0.098	≥6.7	5
SYN548765 (R419492)	56	≥6.28	0.209	≥30.0	5
SYN548766 (R471811)	56	≥5.94	0.197	≥30.2	5
SYN507900	56	≥1.78	0.039	≥45.6	5
R611965	56	≥4.78	0.159	≥30.1	5
R611966	56	≥0.58	0.025	≥23.2	5
R611967	56	≥0.78	0.033	≥23.6	5
R613636	56	≥0.84	0.030	≥28	5

The table above shows that the chronic risk to earthworms is acceptable for chlorothalonil, the product A14111B and the metabolites R182281, R417888, R418503 (tomatoes), R419492, R471811, SYN507900, R611965, R611966, R611967 and R61363, except for the metabolite R418503 for the use in cereals. However, as the NOEC for R418503 is the highest concentration tested in the study and at this concentration no effects at all were observed compared to the control (also no small effects considered not statistically significant), the RMS is of the opinion that at the somewhat higher test concentration needed to reach an acceptable TER, no significant effects are expected. Therefore, the risk is considered acceptable.

B.9.8.2 Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

In Volume 1, section 2.9.4-2 an overview of the available endpoints for non-target soil meso- and macrofauna (other than earthworms) is given.

Ten chlorothalonil metabolites are relevant in soil: R182281 (SDS-3701), R417888 (VIS-01), R418503, R419492, R471811, SYN507900 (SDS-66882), R611965 (SDS-46851), R611966 (SDS-47523), R611967 (SDS-47524) and R613636 (SDS-19221).

However, only for two metabolites toxicity data are available. The notifier has submitted the a statement regarding metabolites of chlorothalonil (see section B.9.8.1). However, the RMS is of the opinion that the argumentation of the notifier regarding the soil metabolites is not sufficiently

convincing. Further argumentation or data on soil metabolites not tested yet, regarding the risk to non-target soil meso- and macrofauna (other than earthworms), is considered necessary.

The notifier submitted chronic toxicity data on the metabolites R418503, R419492, R471811, SYN507900, R611965, R611966, R611967 and R61363.

~~As a conservative approach all endpoints have been divided by 2, irrespective of their log P_{OW} values or whether the tests were conducted in artificial soil containing 5% peat.~~ The highest PECs, whether it was the initial or peak accumulation value has been used for the assessment (for PECsoil values, see section B.8).

The resulting TER_{LT} values are presented below:

Table 9.8.2-01 TER calculations for *Folsomia candida*

Compound	Time scale (days)	NOECcorr (mg a.s./metabolite / kg soil)	Max initial PEC_{soil} (mg as/kg soil)	TER	Trigger
cereals, 2x 750 g a.s./ha, interval 14 days					
BRAVO 500	28	18.2	0.342	53.2	5
A14111B	28	12	0.342	35	5
R182281	28	59.6	0.351	170	5
R417888	28	3.1	0.338	9.1	5
SYN548708 (R418503)	28	≥0.66	0.147	**≥4.49**	5
SYN548765 (R419492)	28	≥6.28	0.314	≥20	5
SYN548766 (R471811)	28	≥5.94	0.297	≥20	5
SYN507900	28	≥1.78	0.056	≥31.8	5
R611965	28	≥4.78	0.239	≥20	5
R611966	28	≥0.58	0.037	≥15.7	5
R611967	28	≥0.78	0.039	≥20	5
R613636	28	≥0.84	0.042	≥20	5
Tomatoes, 1x 1000 g a.s./ha					
BRAVO 500	28	18.2	0.267	68.2	5
A14111B	28	12	0.267	45	5
R182281	28	59.6	0.234	255	5
R417888	28	3.1	0.225	13.8	5
SYN548708 (R418503)	28	≥0.66	0.098	≥6.7	5
SYN548765 (R419492)	28	≥6.28	0.209	≥30.0	5
SYN548766 (R471811)	28	≥5.94	0.197	≥30.2	5
SYN507900	28	≥1.78	0.039	≥45.6	5
R611965	28	≥4.78	0.159	≥30.1	5
R611966	28	≥0.58	0.025	≥23.2	5
R611967	28	≥0.78	0.033	≥23.6	5

R613636	28	≥0.84	0.030	≥28	5

Table 9.8.2-02 TER calculations for *Hypoaspis aculeifer*

Compound	Time scale (days)	NOECcorr (mg a.s./metabolite / kg soil)	Max initial PEC_{soil} (mg as/kg soil)	TER	Trigger
cereals, 2x 750 g a.s./ha, interval 14 days					
R182281	14	≥7.02	0.351	≥20	5
R417888	14	≥6.76	0.338	≥20	5
SYN548708 (R418503)	14	≥0.66	0.147	**≥4.49**	5
SYN548765 (R419492)	14	≥6.28	0.314	≥20	5
SYN548766 (R471811)	14	≥5.94	0.297	≥20	5
SYN507900	14	≥1.78	0.056	≥31.8	5
R611965	14	≥4.78	0.239	≥20	5
R611966	14	≥0.58	0.037	≥15.7	5
R611967	14	≥0.78	0.039	≥20	5
R613636	14	≥0.84	0.042	≥20	5
Tomatoes, 1x 1000 g a.s./ha					
R182281	14	≥7.02	0.234	≥30	5
R417888	14	≥6.76	0.225	≥30.0	5
SYN548708 (R418503)	14	≥0.66	0.098	≥6.7	5
SYN548765 (R419492)	14	≥6.28	0.209	≥30.0	5
SYN548766 (R471811)	14	≥5.94	0.197	≥30.2	5
SYN507900	14	≥1.78	0.039	≥45.6	5
R611965	14	≥4.78	0.159	≥30.1	5
R611966	14	≥0.58	0.025	≥23.2	5
R611967	14	≥0.78	0.033	≥23.6	5
R613636	14	≥0.84	0.030	≥28	5

The tables above show that the chronic risk to *Folsomia candida* and *Hypoaspis aculeifer* are acceptable for chlorothalonil, the product A14111B and the metabolites R182281 and R417888, R418503 (tomatoes), R419492, R471811, SYN507900, R611965, R611966, R611967 and R61363, except for the metabolite R418503 for the use in cereals. However, as the NOEC for R418503 is the highest concentration tested in the study and at this concentration no effects at all were observed compared to the control (also no small effects considered not statistically significant), the RMS is of the opinion that at the somewhat higher test concentration needed to reach an acceptable TER, no significant effects are expected. Therefore, the risk is considered acceptable.

B.9.9 Effects on soil nitrogen transformation

Report:	IIIA, 10.7/01 (numbering in addendum 14 of the DAR (2004)). McMurray, A., 2001b. A laboratory assessment of the effects of Bravo 720 (YF11524) on soil microflora respiration and nitrogen transformations according to current OECD guidelines 216 and 217. Report No. ENV5110 Chemax International plc GLP, Unpublished
Previous evaluation	In addendum 14 to the DAR (2004) for original approval
Remark by RMS	Considered acceptable at the time of original inclusion

Substance	Soil type	Dose [kg/ha][1)]	Dose [mg a.i./kg]	Duration [d]	pF	o.m. [%]	pH	T [°C]	Process	Maximal Effect [%]	After ... [d]	Effect at end [%]
Bravo 720	sandy loam I [2)]	0.0045	3.2	42	2	3.8	6.3	20 ± 2	respiration	-4.49	0	+0.11
Bravo 720	sandy loam I [2)]	0.0045	3.2	42	2	3.8	6.3	20 ± 2	ammonifcation nitrification	+12.25*	14	+3.85
Bravo 720	sandy loam I [2)]	0.0068	4.8	42	2	3.8	6.3	20 ± 2	respiration	+4.26	42	+4.26
Bravo 720	sandy loam I [2)]	0.0068	4.8	42	2	3.8	6.3	20 ± 2	ammonifcation nitrification	+11.98*	14	+6.71
Bravo 720	sandy loam II	0.0045	3.2	28	2	2.2	6.7	20 ± 2	respiration	-13.85	0	-6.03
Bravo 720	sandy loam II	0.0045	3.2	28	2	2.2	6.7	20 ± 2	ammonifcation nitrification	-9.78* [3)]	0	-1.79
Bravo 720	sandy loam II	0.0068	4.8	28	2	2.2	6.7	20 ± 2	respiration	-20.72*	0	-4.13
Bravo 720	sandy loam II	0.0068	4.8	28	2	2.2	6.7	20 ± 2	ammonifcation nitrification	ND		

* Significantly different at P=0.05

[1)] calculated by the RIVM assuming 100% distribution in soil with soil bulk density of 1500 kg/m^3 and 5 cm soil layer

[2)] loamy sand according to author

[3)] –11.31 at day 7 but not significant

Description

The influence of Bravo 720 (53.1% a.i., 701g/L) on soil microflora was determined in a short-term respiration experiment and by monitoring nitrogen transformation according to OECD Guidelines 216 and 217. A dispersion of Bravo 720 in distilled water was applied to a low and a high organic matter sandy loam in a nominal application rate of 6.0 mg Bravo 720/kg, equivalent to 3.2 mg chlorothalonil/kg, and 9.0 mg Bravo 720/kg (4.8 mg chlorothalonil/kg), respectively. Soil was mixed thoroughly. Incubation occurred at 20 ± 2°C. Effects on microbial respiration were determined at day 0, 7, 14, 28 and 42 in three replicates of 1000 g each, by measuring CO_2 evolution in the subsequent 24 hours in aliquots of soil after amending with a non-limiting amount of glucose (0.4g/100g soil dw). Dinoseb acetate was used as a reference compound in a silty sand soil. The effects on nitrogen transformation, ammonification and nitrification were determined by measuring of ammonia-N, nitrate-N, and nitrite-N concentrations in soils amended with ground Lucerne grass within 3 hours after

treatment and after 0, 7, 14, 28 and 42 days, in forty replicates of 50 g each. The concentrations inorganic nitrogen species in the extracts were determined colourimetrically. Soil samples were air dried to allow sieving, then stored at 2-6°C for 2 weeks at moisture content of 21.52% (sandy loam I) and 17.65% (sandy loam II). After preparing samples for respiration and nitrogen transformation, samples were stored at 20 ± 2 °C for 6 days prior to the start of the study. Soil was remoisturised at the start of the experiment to 45 ± 5 % WHC. During the experiment, the soil was remoisturised if necessary. The sandy loam II experiment was restarted (due to inconsistent data) with a further sample of the same soil, which had been refrigerated for 80 days at 2-6^{o}C. Only the lower application rate was investigated for the nitrogen transformation.

Results

Respiration: -4.49%, -3.64%, -4.24%, -1.69 and +0.11% effect was seen for the 6.0 mg/kg treatment and
–1.71%, +1.92%, -1.08%, -1.06% +4.26% for the 9.0 mg/kg treatment after day 0, 7, 14, 28 and 42, respectively, for the high organic soil and –13.85%, +8.63%, -5.0%, 0.0% and –6.03% for the 6.0 mg/kg treatment and
–20.72% (sign. at p= 0.05)), +0.69%, -7.81%, -9.70% and –4.13% for the 9.0 mg/kg treatment for the low organic soil, respectively. The reference compound Dinoseb acetate, produced significant decreases in microbial respiration in a different soil (silty sand soil) of –30.45%, -20.9%, -42.3% and –59.9% at day 0, 7, 14 and 28, respectively.

Nitrogen transformation:
High organic matter sandy loam: Percentage deviation at the lower application rate at zero time was –25.28% (sign. at p=0.05) for ammonium, -0.07% for nitrate, and +1.95% for total mineral nitrogen. At day 7 the percentage deviation was -2.58% for nitrate and total mineral nitrogen, no ammonium could be detected. At day 14 a deviation of –12.25% could be found for both nitrate and total mineral nitrogen, no ammonium could be detected; at day 28 the deviation was –7.9% for both nitrate and total mineral nitrogen, no ammonium could be detected. At day 42 the deviation was –3.85% for both nitrate and total mineral nitrogen, no ammonium could be detected.
Percentage deviation at the higher application rate at zero time was –8.99% for ammonium, -0.41% for nitrate, and –1.10% for total mineral nitrogen. At day 7 the percentage deviation was -2.01% for nitrate and total mineral nitrogen, no ammonium could be detected. At day 14 a deviation of +11.98% (sign. at p=0.05) could be found for both nitrate and total mineral nitrogen, no ammonium could be detected; at day 28 the deviation was +8.53% for both nitrate and total mineral nitrogen, no ammonium could be detected. At day 42 the deviation was +6.71% for both nitrate and total mineral nitrogen, no ammonium could be detected.
The overall rate of nitrate formation between 7 and 42 was calculated by the author to be 1.71 mgN kg soil/day for the control treatment. For the lower application rate a decrease of 8.93% was calculated and for the higher application rate a significant decrease of 13.58%.

Low organic matter sandy loam: Percentage deviation at the lower application rate at zero time was –36.49% (sign. at p=0.05) for ammonium, -6.96% for nitrate, and –9.78% (sign. at p=0.05) for total mineral nitrogen.
At day 7 the percentage deviation was -11.31% for nitrate and total mineral nitrogen, no ammonium could be detected. At day 14 a deviation of –0.74% could be found for both nitrate and total mineral nitrogen, no ammonium could be detected; at day 28 the deviation was –1.79% for both nitrate and total mineral nitrogen, no ammonium could be detected.
The overall rate of nitrate formation between day 0 and 28 was calculated by the author to be 0.42 mgN kg soil/day for the control treatment. For the lower application rate an increase of 15.39% was calculated.

Remarks by RMS

Highest dose was less than ten times the lowest dose. The reference compound was not applied to the soils under study but to a silty sand soil. The soil used for the experiments was derived from a grassland site that had not received pesticide or fertiliser treatment within the last 12 months. It is not clear if the chemically undisturbed period was long enough, i.e. 5 years. The result <25% effect chlorothalonil on the microbial processes soil respiration, ammonification and nitrification after 0, 7, 14, 28 and 42 days at nominal application rates of 6.0 and 9.0 mg BRAVO 720/kg soil, is used for the risk assessment.

Report:	K-CP 10.5/01, Schulz L. (2010), Azoxystrobin/Chlorothalonil SC (A14111B) – Effects on the Activity of Soil Microflora, Report Number 09 10 48 060 C/N, BioChem agrar GmbH, Kupferstraße 6, 04827 Gerichshain, Germany (Syngenta File No. A14111B_10031)
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable. A14111B caused no adverse effects (deviation from control <25%, OECD 217) on soil carbon transformation at 8.07 and 40.37 mg product/kg soil dw (equivalent to 2.57 and 12.8 mg a.s./kg soil dw). Nitrogen transformation rates did not differ from the untreated control by more than 25% at 8.07 mg product/kg soil (equivalen to 2.57 mg a.s./kg soil dw) after 42 days.

Guidelines

OECD guidelines 216 and 217, 2000

GLP: Yes

Executive Summary

A14111B was applied to the soil at concentrations of 8.07 mg product (maximum single concentration expected in the field) and at 40.37 mg product/kg dry soil (five times maximum single concentration expected in the field).

A14111B caused no adverse effects (deviation from control <25%, OECD 217) on soil carbon transformation at 8.07 and 40.37 mg product/kg soil dw (equivalent to 2.57 and 12.8 mg a.s./kg soil dw). Nitrogen transformation rates did not differ from the untreated control by more than 25% at 8.07 mg product/kg soil (equivalen to 2.57 mg a.s./kg soil dw) after 42 days.

Materials

Test Material:	A14111B	
Description:	greyish white	
Lot/Batch No.:	PHY8B80751	
Content of a.i.	azoxystrobin	80 g/L (nominal)
	chlorothalonil	400 g/L (nominal)
	azoxystrobin	78.4 g/L corresponding to 6.47 % w/w
	chlorothalonil	385 g/L corresponding to 31.8 % w/w
Density:	1.211 g/cm³	
Stability:	stable under standard conditions	
Concentrations used:	8.07 and 40.37 mg product/kg soil dry weight (corresponding to 5 and 25 L product/ha, respectively)	
Control:	Deionised water	
Toxic Standard:	Dinoterb (not part of this study)	

Test Design

Soil:	Field soil: Wassergut Canitz (Batch: 3/2009)
Soil type:	Loamy sand (DIN 4220), WHC 38.14 g/100 g soil d.w.
Cultivation:	Fallow ground since 2008, no application of fertilizers since 2003, last application of PPP: 1990.
Soil enrichments:	Nitrogen transformation test: Lucerne meal Carbon transformation test: glucose
Test units:	Nitrogen transformation test: wide-mouth glass flasks (500 mL) Carbon transformation test: stainless steel vessels (4 L)
Replication:	3
Sampling intervals:	0 (3 hours after application), 7, 14, 28 and 42 days (Nitrogen transformation test) 0 (3 hours after application), 7, 14, and 28 days (Cabon transformation test)

Environmental conditions

Temperature:	19.0 – 20.9 °C (Nitrogen transformation test) 19.2 – 20.9 °C (Carbon transformation test)
Photoperiod:	Continuous dark
Soil moisture	45% of maximum water holding capacity

content:	
Soil pH:	Nitrogen transformation test : 6.4 - 6.6, Carbon transformation test: 6.6
Duration of test:	42 days (Nitrogen transformation test) 28 days (Carbon transformation test)

Study Design and Methods

Experimental dates: 28th October to 9th December 2009

Soil samples were treated with A14111B at two doses – 8.07 mg product/kg (low dose) and 40.37 mg product/kg dry soil (high dose). These represent once and five times the field rate, based on the maximum single application rate of 5 L product/ha with one application/year (soil depth of 5 cm and soil density of 1.5 g/cm^3)

A stock solution of the product was prepared with deionised water, which was added to the soil samples and mixed thoroughly. The soil moisture content of all samples was adjusted to 45 % of the MWC by adding deionised water and the samples incubated in the dark at a temperature of 20 ± 2°C. The soil moisture content was checked weekly, and adjusted with deionised water to maintain 45 % of the soil MWC.

Respiration was determined for all treatments at 0 (3 hours), 7, 14 and 28 days after treatment using a respirometer Nitrification was determined for all treatments at 0 (3 hours), 7, 14 and 28 days after treatment. Due to measured deviations of > 25 % observed in the treatment group treated with 40.37 mg/kg soil dry weight 28 days after application, the test had to be prolonged up to day 42 after application. To determine the nitrification, the soil samples were amended with lucerne meal before application and triplicate samples were taken at each sampling occasion. The samples were extracted with KCl, and analysed for nitrite-nitrogen and nitrate-nitrogen.

Statistical analysis was performed with the software ToxRat Professional 2.10 (RATTE 2009). The Student-t-test (two-sided, α = 0.05) for homogeneous variances as pair-wise comparison of treatments with “Control” were used.

According to the guideline, evaluation should be based on transformation rates in consecutive intervals (i.e. day 0-7, day 7-14 etc), rather than for each sampling from day 0 (i.e. day 0-7, day 0-14 etc). Transformation rates for consecutive intervals were calculated by RMS.

Results and Discussion

The results for the respiration and nitrification are summarised below.

Table 10.5-4: Effects of A14111B on glucose-induced short-term respiration

Days after application	**Control**	**8.07 mg product/kg soil dry weight equivalent to 5 L product/ha**		**40.37 mg product/kg soil dry weight equivalent to 25 L product/ha**	
	O_2	O_2 consumption	Deviation from	O_2 consumption	Deviation from

	consumption [mg/kg soil d.w./h]	[mg/kg soil d.w./h]	control [%][1]	[mg/kg soil d.w./h]	control [%][1]
0	10.27	9.66*	-6.0	8.56*	-16.6
7	8.99	8.79*	-2.2	6.92*	-23.0
14	8.75	8.68	-0.7	6.79*	-22.4
28	8.78	8.48*	-3.4	7.08*	-19.3

The calculations were performed with non-rounded values.

1) based on O_2 consumption; - = inhibition; + = stimulation

* statistically significantly different from control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Table 10.5-5: Effects of A14111B on nitrate formation in the soil

Treatment rate (mg product/kg soil dw)	**Nitrate levels (mg/kg soil dw) on day**				
	0	**7**	**14**	**28**	**42**
Control	14.5	28.6	29.9	37.3	41.1
8.07	14.3	32.5	31.1	39.6	43.2
40.37	14.6	42.2	47.3	51.6	50.8
	% deviation (nitrate levels) from control				
8.07	-1	+14	+4	+6	+5
40.37	+1	+48	+59	+38	+24
	% deviation (nitrate formation rate) from control on day				
	0-7		**7-14**	**14-28**	**28-42**
8.07	+29		-208	+15	-5
40.37	+96		+292	-42	-121

Absolute nitrate levels at 8.07 mg product/kg soil dw differed from untreated soil by ≤14% at any sampling and nitrification rates were not affected by more than 15% after 14 days. Therefore, no long-term effects on soil nitrification were observed at this concentration.

At 40.37 mg product/kg soil dw, absolute nitrate levels differed by 24% at the end of the 42-day exposure period. Nitrate transformation rates differed from untreated soil by ≥42% for all sampling intervals. Therefore, the study should have been prolonged until transformation rates differed from those in untreated soil by less than 25% or until 100 days, whichever was reached earlier.

Validity criteria

The coefficients of variation in the control group of the nitrogen and carbon transformation tests were maximum 4.6 % and 1.8 %, respectively (demanded range ≤15 %).

Reference substance Carbon transformation test

In the most recent test, dated 08.01. to 05.02.2009, the toxic standard dinoterb caused effects of -28.8 %, -42.1 %, and -46.9 % (required ≥ 25 %) on the carbon transformation in a field soil at the tested concentrations of 6.80 mg, 16.00 mg and 27.00 mg dinoterb per kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Reference substance Nitrogen transformation test

In the most recent test, dated 08.01. to 05.02.2009 the toxic standard dinoterb caused effects of + 37.9 and +48.3 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg Dinoterb kg soil dry weight, respectively, 28 days after application and thus demonstrates the sensitivity of the test system.

Conclusions

A14111B caused no adverse effects (deviation from control <25%, OECD 217) on soil carbon transformation at 8.07 and 40.37 mg product/kg soil dw (equivalent to 2.57 and 12.8 mg a.s./kg soil dw).. Nitrogen transformation rates did not differ from the untreated control by more than 25% at 8.07 mg product/kg soil (equivalen to 2.57 mg a.s./kg soil dw) after 42 days.

B.9.10 Risk assessment for soil nitrogen transformation

In Volume 1, section 2.9.4-2 an overview of the available endpoints for soil microbial processes is given.

Ten chlorothalonil metabolites are relevant in soil: R182281 (SDS-3701), R417888 (VIS-01), R418503, R419492, R471811, SYN507900 (SDS-66882), R611965 (SDS-46851), R611966 (SDS-47523), R611967 (SDS-47524) and R613636 (SDS-19221).

However, only for three metabolites effect data are available. The notifier has submitted the a statement regarding metabolites of chlorothalonil (see section B.9.8.1). However, the RMS is of the opinion that the argumentation of the notifier regarding the soil metabolites is not sufficiently convincing. Further argumentation or data on soil metabolites not tested yet, regarding the risk to soil nitrogen transformation, is considered necessary.

The notifier submitted chronic toxicity data on the metabolites R418503, R419492, R471811, SYN507900, R611965, R611966, R611967 and R61363.

The table below gives an overview of the available data on soil nitrogen transformation:

Table 9.10-01: Overview of available data for effects on soil nitrogen transformation

Test substance	Effects ≤ 25% at test concentration (expressed in mg a.s./kg dry soil):
Chlorothalonil	10.0
A14111B	2.57
R182281 (SDS-3701)	1.3

R611965 (SDS-46851)	1.25 and 2.5
R417888	2.48 and 4.96
SYN548708 (R418503)	≥0.66
SYN548765 (R419492)	≥6.28
SYN548766 (R471811)	≥5.94
SYN507900	≥1.78
R611966	≥0.58
R611967	≥0.78
R613636	≥0.84

The exposure to soil organisms was estimated by calculating the maximum instantaneous predicted environmental concentrations in soil (PEC_{Soil}) as presented in Section B.8. The PEC_{soil} values are repeated below for convenience.

Table 9.10-02: Summary of initial PEC_S of A14111B, chlorothalonil and its soil metabolites, to cereals at 2 x 750 g/ha, BBCH 30-69 and tomatoes at 1 x 1000 g/ha, BBCH 51-89

Formulation/ compound	Crop/use pattern	$PEC_{S, initial}$ [mg/kg]	$PEC_{S, plateau}$ [mg/kg]	$PEC_{S, peak\ accum}$ [mg/kg]
A14111B [a]	2 x 750 g a.s./ha cereals	**0.342**	-	-
Chlorothalonil		**0.342**	-	-
R182281		0.118	0.233	**0.351**
R417888		0.075	0.263	**0.338**
R611965		0.066	0.186	**0.239**
SYN548708 (R418503)		0.033	0.114	**0.147**
SYN548765 (R419492)		0.066	0.244	**0.314**
SYN548766 (R471811)		0.066	0.231	**0.297**
SYN507900		0.02	0.036	**0.056**
R611966		0.029	0.0077	**0.037**
R611967		0.039	-	**0.039**
R613636		0.042	-	**0.042**
A14111B [a]	1 x 1000 g a.s./ha tomatoes	**0.267**	-	-
Chlorothalonil		**0.267**	-	-
R182281		0.079	0.154	**0.234**
R417888		0.050	0.174	**0.225**
R611965		0.036	0.123	**0.159**
SYN548708 (R418503)		0.022	0.0759	**0.098**
SYN548765 (R419492)		0.047	0.162	**0.209**
SYN548766 (R471811)		0.044	0.153	**0.197**
SYN507900		0.015	0.024	**0.039**

Formulation/ compound	Crop/use pattern	$PEC_{S, initial}$ [mg/kg]	$PEC_{S, plateau}$ [mg/kg]	$PEC_{S, peak\ accum}$ [mg/kg]
R611966		0.020	0.0050	**0.025**
R611967		0.033	-	**0.033**
R613636		0.030	-	**0.030**

Looking at the PECsoil values it is clear that these values are below the concentrations in soil which have acceptable effects on soil nitrogen transformation. Hence, the risk from chlorothalonil, the product A14111B and the metabolites is acceptable.

B.9.11 Effects on terrestrial non-target higher plants

B.9.11.1 Summary of screening data

A summary of a study conducted with the representative formulation is presented below.

Report: K-CP 10.6.1/01 Walder, L. (2004). Herbicide profiling test to evaluate the phytotoxicity of azoxystrobin/chlorothalonil 480 EC (A14111B) to terrestrial non-target plants. Report Number SMQ 03010. Syngenta Crop Protection AG, Stein, Switzerland. (Syngenta file No. ICI5504/2161)	
Previous evaluation	Submitted for the purpose of renewal (new study)
RMS remark	Acceptable A14111B did not cause any adverse effects on 6 plant species at treatment rates up to and including 2500 mL/ha. This non-GLP study was not conducted according to a guideline, but study procedures were generally in agreement with OECD 208 and 227. Data for the controls were however not reported and therefore the validity of the study could not be evaluated. Therefore, the result of this study can be used in a weight-of-evidence approach only.

Guidelines

In-house SOP, Basic Herbicide Profiling Test.

GLP: No. The study was performed according to sound scientific practices.

Executive Summary

The test item was sprayed pre- and post-emergence to potted plants in the greenhouse. Two monocotyledonous (wild oat *Avena fatua*, onion *Allium cepa*) and four dicotyledonous (cucumber *Cucumis sativus*, sugar beet *Beta vulgaris*, oilseed rape *Brassica napus*, soybean *Glycine max*) species were used as test plants. Application rates were 0, 0.078, 0.156, 0.313, 0.625, 1.25 and 2.5

L/ha. In the seedling emergence test, test units were treated after sowing the seeds that had been watered for 24 hours, and maintained for 28 days under controlled conditions. In the vegetative vigour test, plants were grown for 14 to 19 days prior to application and for 21 days after application of the test item.
A14111B caused no observable effects in any tested plant species at treatment rates up to and including 2500 mL/ha.

Materials

Test Material:	Azoxystrobin/chlorothalonil SC (80/400) (formulation code A14111B)
Description:	Opaque cream-coloured liquid, nominally containing 80 g/L azoxystrobin and 400 g/L chlorothalonil
Lot/Batch #:	J7518/024
Purity:	Not reported, but from other study reports (e.g. K-CP 10.3.1.1.1/01): 80 g/L azoxystrobin (6.6 % (w/w)) and 419 g/L chlorothalonil (34.6 % (w/w))
Stability of test compound:	Not reported, but from other study reports (e.g. K-CP 10.3.1.1.1/01): assumed stable pending re-analysis in September 2005
Test organisms	
Species:	*Avena fatua*, *Allium cepa*, *Cucumis sativus*, *Beta vulgaris*, *Brassica napus*, *Glycine max*
Environmental test conditions	
Temperature:	Minimum day/night: 20°C / 15°C
Humidity:	40 - 60% relative humidity
Photoperiod:	14 hours light / 10 hours dark

Study Design and Methods

Experimental dates: 17th December 2003 to 14th January 2004.
The test item was sprayed pre- and post-emergence to potted plants in the greenhouse. Two monocotyledonous (wild oat *Avena fatua*, onion *Allium cepa*) and four dicotyledonous (cucumber *Cucumis sativus*, sugar beet *Beta vulgaris*, oilseed rape *Brassica napus*, soybean *Glycine max*) species were used as test plants. Application rates were 0, 0.078, 0.156, 0.313, 0.625, 1.25 and 2.5 L/ha. Each treatment was tested in duplicate. Depending on the plant species, between 3 and 20 seeds were used per test unit (non porous 10-cm-deep plastic trays with perforated bottom). The soil used was a clay loam from local origin (26 % clay, 34 % silt, 40 % sand, 2.6 % organic matter and pH 7.5). Treatments were applied with a laboratory sprayer, set to deliver an output of 500 L/ha. In the seedling emergence test, test units were treated after sowing the seeds that had been watered for 24 hours, and maintained for 28 days under controlled conditions. Plants used in the vegetative vigour test were grown for 14 to 19 days prior to treatment (2 to 4 leaves growth stage) and afterwards

maintained under controlled conditions for another 21 days. Plants were watered from the top of the trays according to needs, and nutrients were supplied twice a week using a commercial fertiliser. Temperature during the test ranged from 15 to 22 °C. The relative humidity was 40 to 60% for all species, and a 14-hour photoperiod (min. 10000 lux) per day was maintained. At the test end, phytotoxicity was assessed according to a visual scale ranging from 0 (= no visual damage, normal growth) to 100 (= complete kill/no emergence), always as compared to the untreated control.

Results and Discussion

The results are summarised in Table 10.6.1-1.

Table 10.6.1-1: A14111B - Effects on non-target plants

Plant species / family	**Seedling emergence**						**Vegetative vigour**					
Application rate (mL A14111B/ha) :	2500	1250	625	312.5	156.3	78.13	2500	1250	625	312.5	156.3	78.13
B. napus / Cruciferae	0	0	0	0	0	0	0	0	0	0	0	0
A. fatua / Gramineae	0	0	0	0	0	0	0	0	0	0	0	0
B. vulgaris / Chenopodiaceae	0	0	0	0	0	0	0	0	0	0	0	0
C. sativus / Cucurbitaceae	0	0	0	0	0	0	0	0	0	0	0	0
G. max / Leguminosae	0	0	0	0	0	0	0	0	0	0	0	0
A. cepa / Allioideae	0	0	0	0	0	0	0	0	0	0	0	0

Rating scale: 0 = no visual damage; 100 = no emergence or complete destruction of plant parts above ground; data are the average of 2 replicates and are in comparison to the controls

Conclusion

A14111B did not cause any adverse effects on 6 plant species at treatment rates up to and including 2500 mL/ha.

Remarks RMS

This non-GLP study was not conducted according to a guideline, but study procedures were generally in agreement with OECD 208 and 227. Data for the controls were however not reported and therefore

the validity of the study could not be evaluated. Therefore, the result of this study can be used in a weight-of-evidence approach only.

B.9.7.3 Testing on non-target plants

Further testing is not required since A14111B does not exhibit herbicidal activity.

B.9.11.2 Extended laboratory studies on non-target plants

Extended laboratory tests were not conducted as the risk assessment below indicates acceptable risk to non-target plants.

B.9.11.3 Semi-field and field tests on non-target plants

Semi-field or field tests were not conducted as the risk assessment below indicates acceptable risk.

B.9.12 Risk assessment for terrestrial non-target higher plants

Toxicity

The effect of A14111B on seedling emergence and vegetative vigour in 6 plant species was evaluated in a glasshouse study (***Wälder, 2004***). Pre- and post-emergence applications of A14111B at rates up to and including 2500 mL/ha did not have an adverse effect on seedling emergence or subsequent shoot growth. Further details of the study are provided under CP 9.11.1 above. However, data for the controls were not reported and therefore the validity of the study could not be evaluated. Therefore, the result of this can be used in a weight-of-evidence approach only.
Taking the results of the study with the active substance into account (see CA-B.9.6.1), a NOER value of 18 kg as/ha was found, it is clear that chlorothalonil is not toxic for non-target plants. A risk assessment, based on the results of the test with the formulation is performed below.

Exposure

Effects on non-target plants are of concern in the off-crop environment, where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the **BBA (2000)**[23] from the spray-drift predictions of ***Ganzelmeier & Rautmann (2000)***[24]. Only a single application is considered as factors such as plant growth will reduce residues per unit area between multiple applications. For a single application of A14111B, 2.77 % of the in-field application rate is assumed to reach areas at a minimum distance of 1 m from the edge of the crop for cereals. For tomatoes this drift percentage is 8.02% (default for vegetables higher than 50 cm at 3 m distance, 90th percentile).
For tomatoes, the single application rate of A14111B is 2500 mL product/ha, giving a maximum off-crop predicted environmental rate ($PER_{off\text{-}crop}$) of 200.5 mL A14111B/ha.

[23] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.
[24] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

For cereals, the single application rate of A14111B is 1875 mL product/ha, giving a maximum off-crop predicted environmental rate ($PER_{off-crop}$) of 51.9 mL A14111B/ha.

Risk assessment

A1411B

A14111B is a fungicide and is therefore not expected to have any significant herbicidal activity. A profiling study of the effects on pre- and post-emergence non-target higher plants was conducted with the formulation A14111B. On any of the six species tested at 2500 mL formulation/ha no adverse effects on seedling emergence or subsequent shoot growth were observed. The respective risk assessment is provided below.

Table 9.12-1: TER values for non-target plants

Crop/use pattern	Endpoint	PER	TER	Trigger
Vegetative vigour / seedling emergence				
1 x 1000 g a.s./ha tomatoes	ER_{50} >2500 mL A14111B./ha	200.5 mL formulation/ha	>12.5	5
2 x 750 g a.s./ha cereals		51.9 mL formulation/ha	>48	

The estimated maximum $PER_{off-field}$ values are clearly below the level found to have no effects on the non-target plants. The risk assessment is considered to be sufficiently convincing for the conclusion that the risk of A14111B to non-target plants is low.

Metabolite SDS-3701

In the CA-document a study with the metabolite SDS-3701 is presented. A soil application of SDS-3701 at rates up to 7500 g SDS-3701/ha resulted in ER_{50} values ranging from 143 to >7500 g SDS-3701/ha. SDS-3701 reduced shoot weight and inhibited emergence of all species at the tested rates except emergence of *Avena sativa*. *Allium cepa* was the most sensitive species, with an ER_{50} of 143 g SDS-3701/ha, based on biomass. The risk assessment based on this value is presented in table B.9.12-2:

Table B.9.12-2: Risk assessment of metabolite SDS-3701 to non-target plants (exposure by soil)

Crop	Appl. rate (g a.s./ha)	PECsoil in-crop (mg/kg soil)	% Drift	PECsoil off-crop (mg/kg soil)	ER_{50} (mg/kg soil)	TER
Potato	750 (1x)	0.131	2.77	0.004	0.19	48
Cereals	750 (2x)	0.351	2.77	0.01	0.19	19
Tomato	1000 (1x)	0.234	8.02	0.019	0.19	10

The resulting TER values are above the trigger value of 5.
Therefore, the metabolite SDS-3701 poses no unacceptable risk to terrestrial non-target plants in off-crop areas following the proposed use.

Conclusion

When applied in accordance with the uses supported in this submission A14111B does not pose an unacceptable risk to non-target plants, based on the indicatieve risk assessment performed above and taking into account the results of the screening test with the active substance alone.

B.9.13 Effects on other terrestrial organisms (flora and fauna)

No further data on other terrestrial organisms is required.

B.9.14 Risk assessment for other terrestrial organisms (flora and fauna)

No further risk assessments on other terrestrial organisms are required.

B.9.15 References relied on

The ecotoxicology reference list and references in the text must be re-numbered due to many repeated numbers in the original DAR, the various addenda, and this RAR. This will be completed shortly and the RAR updated.

KCA 8.4.2.1 / 01	Vinall S.	2014	Chlorothalonil SC (A7867A) - A laboratory test to determine the effects of fresh residues on the predatory mite Hypoaspis aculeifer (Acari, Laelapidae) Syngenta Mambo-Tox Ltd., Southampton, United Kingdom, SYN-14-1 GLP not published Syngenta File No A7867A_11243	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Chlorothalonil Task Force